Original article

Pro j 2 is mesquite profilin: molecular characteristics and specific IgE binding activity

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Summary

Background: **Pollens from mesquite (*Prosopis juliflora*) are potent allergen responsible in causing immediate hypersensitivity reactions in susceptible people in tropical countries.**

Objective: **This study aimed to clone, express and purify the mesquite pollen profilin (Pro j 2) as well as evaluating its nucleotide sequence homology in order to predict allergenic cross-reactivity with profilins of common allergenic plants.**

Methods: **Immunoblotting assay and specific ELISA were applied to determine the immunoreactivity of sera from 35 patients who were allergic to mesquite pollen. The mesquite profilin-coding sequence was cloned into PTZ57R/T vector and amplified. The cDNA of mesquite pollen profilin was then expressed in *Escherichia coli* using pET-21b (+) vector and purified by one-step Ni^{2+} affinity chromatography. IgE binding capacity of the recombinant mesquite profiling (rPro j 2) was analyzed by specific ELISA, immunoblotting, and inhibition assays.**

Results: **cDNA nucleotide sequencing revealed an open reading frame of 399bp encoding for 133 amino acids which belongs to the profilin family. Seventeen patients (17/35, 48.57%) had significant specific IgE level for rPro j 2. Immunodetection and inhibition assays indicated that purified rPro j 2 might be similar as that in the crude extract.**

Conclusion: **Pro j 2, as a new allergen from mesquite pollen, was produced in *E. coli* with an IgE-reactivity similar to that of its natural counterpart. The amino acid sequences homology analysis of mesquite profilin and several profilin molecules from other plants showed high degree of cross-reactivity among plant-derived profilins from unrelated families.** ***(Asian Pac J Allergy Immunol 2015;33:90-8)***

Keywords: *Mesquite, profilin, Pro j 2, cloning, expression*

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Introduction

Immediate type allergies represent an important health problem in industrialized countries. One approach to develop deeper understanding of the pathogenesis of allergic diseases is to improve our knowledge of the structure and biological function of atopic allergen using recently developed molecular biological techniques.[1]

High exposure to allergens including pollens is increasingly reported as an important determinant of important allergic diseases such as asthma, and rhinoconjunctivitis. Climate change, and the environmental disturbance it implies, may result in an increase of the allergen burden in the environment, and when combined with higher pollutant levels, can significantly increase respiratory symptoms and allergic responses in the population. The patterns of sensitization to pollens depend on exposure risks which vary depending on the environmental characteristics (climate, geography, vegetation, etc.) of a given region, making predictions of allergy occurrence difficult.[2]

The mesquite, *Prosopis juliflora* (Fabaceae), has been identified as an important source of pollen allergens. The mesquite is native from arid regions of Southwestern United States and Mexico but has now acclimatized to various regions in Asia and the States of the Indian sub-continent. Mesquite flowers are produced year-long (Feb–May; Aug–Nov).[3]

The mesquite is an important cause of allergic disease in tropical countries, including the Saudi

Arabia, South Africa,[4,5] Kuwait,[6] United Arab Emirates (UAE),[7] India,[8]and Southwestern United States.[9,10] Where it has been introduced, the mesquite has been planted as a street tree in towns and ornamental shade tree in parks and gardens. Due to their very deep root system, mesquite trees need only little or no watering and can survive in regions with limited rainfall.[4,8]

Previous studies have shown mesquite tree pollen to be an important allergen source in tropical and subtropical countries, with proteins of 14–97 kDa as IgE binding components.[3,4,9,11] Further proteins of 14, 41, 52 and 66 kDa were recognized as shared allergens among mesquite and other tree pollens.[3] Immunoblotting with pooled patients' sera demonstrated 16 IgE binding components, with components of 14, 24, 26, 29, 31, 35, 52, 58, 66 and 95 kDa recognized by more than 80% of individual patients' sera.[3]

Until now, different MWs have been reported for profilins from different plant sources, such as 14.3 KDa in *Salsola kali* pollen,[12] and 14.4 KDa *Chenopodium album* pollen.[13] It was suggested that the 14-15kDa protein band belongs to the profilin family and two degenerate primers, which hybridized with a DNA whose sequence derived from that of the conserved sequences of profilin family were used in this study.

Since the discovery of their allergenicity in 1991, profilins were identified as allergen from many fruits, vegetables, foods, as well as pollen.[14] Profilins are highly conserved with sequence similarity ranging from 70 to 80% among known plant profilins.[15] Profilin was first recognized as an allergen in birch pollen and named Bet v 2. However, these allergens are usually under-represented in the natural extracts used for diagnostic, therapeutic and experimental investigations.

Since mesquite pollen allergens play an important role in sensitization of allergic patients, the present experiment was carried out to clone, express and purify mesquite pollen profilin using *Escherichia coli* (*E.coli*) as a vector and evaluate its nucleotide sequence homology with other plant-derived profilins (Sal k 4, Ama r 2, Hev b 8, Ole e 2, Che a 2) to predict allergenic cross-reactivity patterns. *Prosopis juliflora* pollen profilin, as a novel allergen, was designated Pro j 2 by the WHO/IUIS Allergen Nomenclature Subcommittee.

Methods

Allergic patients and skin prick test (SPT)

The study included 35 patients suffering from allergy to mesquite pollen, 20 men and 15 women who had seasonal rhinitis without asthma. All the subjects had positive SPT to mesquite pollen extract. Six other healthy subjects who showed negative SPT responses and no specific IgE to mesquite pollen extract were used as negative control. The Human Ethics Committee of the institute approved the study protocol with informed written consent obtained from each patient.

Pollen extract preparation

Polleniferous material was collected from mesquite's flowers during February-April throughout Ahvaz city, a tropical region in southwest Iran with a tropical climate and a population of more than 1.4 million.[16]

Collection and processing of pollen materials was done carefully by trained pollen collectors. Pollen grains were separated by passing the dried materials through different sieves (100, 200 and 300 meshes) successively. The final product, a fine powder, was subjected to a purity check for pollen content using a microscope. Pollen materials with more than 95% pollen and less than 5% floral parts of the same plant were taken for protein extraction.

Pollen materials were defatted using repeated changes of diethyl ether. Pollen was extracted as described previously.[17] In brief, two grams of pollen was mixed with 10ml phosphate-buffered saline (PBS) 0.01 M (pH 7.4) by continuous stirring for 18 hours at 4˚C. The supernatant was separated by centrifugation at 14,000 g for 30 min, filtered and the supernatant collected. The extract was then freeze-dried. The protein content of the extract was measured using the Bradford's method.[18]

Total IgE and Specific enzyme- linked immunosorbent assay (ELISA)

Total serum IgE levels were determined using a commercial ELISA kit following the manufacturer's instructions (Euroimmun, Lübeck, Germany).

To measure the levels of specific IgE in response to mesquite pollen exposure in patients' sera, an indirect ELISA was developed as described previously.[19] In brief, 0.1 µg of mesquite pollen extract in 100 µl carbonate buffer (15 mM Na_2CO_3 and 35 mM $NaHCO_3$, pH 9.6) was incubated at

4 °C overnight per well of a 96-well microtiter plate (Nunc A/S, Roskilde, Denmark). Each well was then blocked for 1 hour at 37°C with 150 µl of 2% BSA in PBS followed by incubation for 3 hours with 100 µl of serum at room temperature with shaking. Each well was then incubated for 2 hours at room temperature with 1:1000 dilution of biotinylated goat anti-human IgE antibody (Nordic-Mubio, Susteren, Netherlands) in PBS. Each incubation step was followed by five washes with TPBS. Wells were complemented by 100µl of a 1:5000 dilution of horseradish peroxidase-conjugated streptavidin (Bio-Rad, Hercules, CA, USA). Following five washes, 100µl of chromogenic substrate was added to each well and the plate was incubated for 15 min in the dark. The plate was read at 450 nm with an ELISA reader. Optical density (OD_{450}) greater than four times the median values of the negative controls were considered to be positive.

Amplification of Pro j 2 cDNA and determination of nucleotide sequence

Total RNA, isolated from 100mg of mesquite pollen was obtained using the method of Chomczynski and Sacchi.[20] First strand of cDNA was synthesized using RevertAid™ First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA) following the manufacturers' instructions. Degenerate primers for cDNA amplification were designed according to consensus sequence for plant pollen profilins. The primers used were the sense 5′-ATGTCSTGGCAGACGTAYGT HGATGA-3′ and the antisense5′-CATGCCYTGTT CGACCAGRTARTCACC-3′. The amplified product was ligated into the PTZ57R/T TA cloning vector from InsTAclone™ PCR Cloning Kit (Thermo Scientific, Waltham, MA, USA), following the manufacturer's instruction. *Escherichia coli* TOP 10 cells (Invitrogen, Carlsbad, CA, USA) were transformed with the ligation products following the manufacturer's protocol. Recombinant plasmid was then purified from the gel using a Plasmid Extraction Kit (GeNet Bio, Chungnam, Korea) and sequenced by the dideoxy method and analyzed at the SeqLab Sequence laboratories (Gttingen, Germany).

Construction of prokaryotic expression plasmid carrying Pro j 2gene

The coding region from Pro j 2 was amplified with *Pfu* DNA polymerase (Thermo Scientific, Waltham, MA, USA), using two specific primers. The obtained sequence (GenBank accession number: KJ462467) allowed for the design of specific primers for Pro j 2 cDNA. These primers contained overhangs with *Not* I and *Xho* I restriction sites allowing direct cloning into expression plasmid pET-21b(+) (Novagen, Gibbstown, NJ, USA) as follow: The sense primer (5′-TCCGCGGCCGC AATGTCCTGGCAGACGTATGTAGA-3′, *Not* I restriction site underlined) and the antisense primer(5′-CCCTCGAGCATGCCTTGTTCGACCA GATAGT-3′, *Xho*I restriction site underlined).

After PCR amplification, the resulting product was digested with *Not* I and *Xho* I restriction enzymes according to the manufacturer's protocol (Thermo Scientific, Waltham, MA, USA). The purified digested PCR product was ligated into the digested pET-21b(+) plasmid with the same enzymes. Correct constructs were transformed into competent *E. coli* BL21 (DE3) cells (Novagen, Gibbstown, NJ, USA).

Expression and purification of recombinant mesquite profilin (rPro j 2)

A fresh clone of recombinant plasmid pET-21b(+)/Pro j 2 was inoculated into 2ml of LB medium containing 100 µg/ml of ampicillin and was incubated at 37˚C. Expression of the recombinant protein was induced by adding isopropyl β-D-thiogalactopyranoside(IPTG) at the concentration of 0.2mM[21]. After induction, in order to improve the solubility of rPro j 2, the cultures were incubated at 20˚C and the cells were allowed to grow for a period of 12 hours. The cells were subsequently harvested by centrifugation (3,000 Xg, 15 min, 4˚C), resuspended in lysis buffer (50mM Tris–HCl pH 6.8, 15mM imidazole, 100mM NaCl, 10% Glycerol, and 0.5% Triton X-100), and subjected to three freeze-thaw cycles with liquid nitrogen. Purification of rPro j 2 was performed with Ni-NTA agarose (Invitrogen, Carlsbad, CA, USA) from the soluble phase of the lysate, following the manufacturer's instructions.

Specific ELISA for rPro j 2 and ELISA inhibition assay

In order to quantify the serum specific IgE levels in reaction to the purified rPro j 2 exposure, an indirect ELISA was developed following the same procedure as described above with the exception that the wells of the ELISA microplate were coated with 15µg per well of the purified rPro j 2.

ELISA inhibition assays were performed as described above, except that a pooled serum (1:2

v/v) from hypersensitive mesquite allergic patients (Nos. 1, 2, 8, 10, 11 and 14) was pre-incubated overnight at 4°C with either 1000,100, 10, 1, 0.1 or 0.01 µg of rPro j 2 as inhibitors or with BSA as a negative control. Percentage of inhibition was calculated using the following formula: (OD of sample without inhibitor - OD of sample with inhibitor / OD of sample without inhibitor) ×100.

IgE-Immunoblotting and IgE-immunoblotting inhibition

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) of mesquite pollen extract was performed according to Laemmli[22] using 12.5% acrylamide separation gels under reducing conditions. The molecular masses of protein bands were estimated with Image Lab Analysis Software (Bio-Rad, Hercules, CA, USA) by comparison with protein markers of known molecular weights (Amersham Low molecular weight Calibration Kit for SDS electrophoresis, GE Healthcare, Little Chalfont ,UK). Separated protein bands from the electrophoresis of mesquite pollen extract were electro-transferred to polyvinylidene difluoride (PVDF) membranes (GE Healthcare, Little Chalfont, UK), as described earlier.[17] In brief, after blocking and washing, membranes were incubated with a serum pool or individual sera from patients with mesquite allergy or with control sera (1:5 dilutions) for 3 hours. Biotinylated goat anti-human IgE (Nordic-Mubio, Susteren, Netherlands) (1:500 v/v in PBS) was added to the blotted membrane strips and incubated for 2 hours at room temperature. The unbound antibodies were removed from blots by washing with TPBS (PBS containing 0.05% Tween 20) and followed by incubation with 1:10,000 v/v in TPBS-HRP- linked streptavidin (Sigma-Aldrich, St. Louis, Mo, USA) for 1 hour at room temperature.

After several washes with TPBS, strips were incubated with Supersignal West Pico Chemiluminescent Substrate Kit (Thermo Scientific, Waltham, MA, USA) for 5 min, and proteins were then visualized by chemiluminescence using ChemiDoc XRS+ system (Bio-Rad, Hercules, CA, USA).

To study cross-inhibition between natural and rPro j 2, a mixture of 100 µl of pooled serum (1:5 v/v) was incubated with natural mesquite pollen extract (20 µg/ml, as inhibitor), rPro j 2 (10 µg/ml, as inhibitor), or BSA (as negative control) overnight at 4˚C with shaking. Pre-incubated sera were used to assess the reactivity of a PVDF membrane blotted with natural mesquite pollen extract and rPro j 2, as described above.

Results

Patients, SPT, Total and specific IgE levels

Thirty five allergic patients, 20 men and 15 women (mean age, 31.51±9.92 years; age range 16-56 years), were included in the present study. All patients were positive for SPT using mesquite pollen extract (mean diameter of weal, 7.86±2.64 mm; diameter range 5-15 mm). The mean total IgE serum was determined as 291.49±140.49 IU/ml (Range 123-679 IU/ml). Specific IgE profiles were determined by ELISA for mesquite pollen extract in allergic patients (Table 1). All patients showed elevated specific IgE (Mean of OD_{450}, 1.61±0.44; OD_{450} range 0.85-2.71).

IgE-binding components of mesquite pollen extract

The reducing SDS-PAGE separation of pollen extract showed several protein bands in the *P. juliflora* pollen extract with molecular weights ranging from 10 to 95 kDa (Figure 1, lane 1). The most prominent bands had MWs of approximately 15, 18, 45 and 85 kDa.

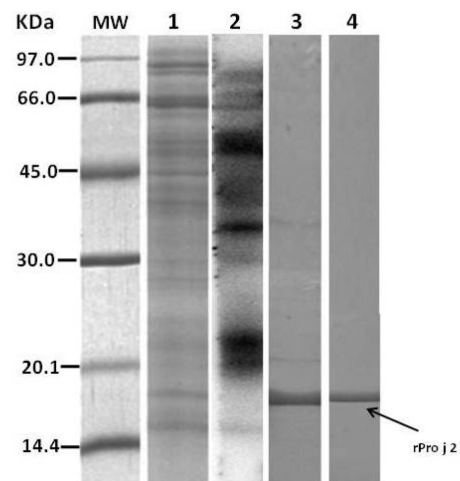

Figure 1. SDS–PAGE and immunoreactivity of mesquite pollen extract and recombinant mesquite profilin (rPro j 2).**A.** Lane MW, molecular weight marker (GE Healthcare, Little Chalfont, UK); lane 1: Coomassie Brilliant Blue stained SDS–PAGE of the crude extract of mesquite pollen; lane 2: Immunoblotting of mesquite pollen extract. The strip was first blotted with mesquite pollen extract and then incubated with polled sera of mesquite allergic patients (n=12) and detected for IgE reactive protein bands; lane3:rPro j 2 in soluble fraction; lane 4: purified rPro j 2 (as an approximately 18-kDa recombinant protein) on 12.5% acrylamide gel.

Immunoblotting experiments using mesquite pollen extract with pooled sera from SPT and specific ELISA positive patients (n=12) showed several IgE reactive bands ranging from about 10 to 85 kDa (Figure1, lane 2). While the most frequent IgE reactive bands among the patients' sera were approximately 20 and 66kDa, there were other IgE reactive protein bands among patients' sera with the following molecular weights 10, 15, 35, 45, 55 and 85kDa (Figure 1, lane 2).

Cloning and sequence analysis of Pro j 2

The sequence analysis of Pro j 2 showed an open reading frame of 399bp coding for 133 amino acid residues with a predicted molecular mass of 14.165 kDa and a calculated pI of 4.73. The obtained nucleotide sequence was submitted to NCBI GenBank (Accession Number: KJ462467) (Figure 2).

The comparison of the deduced amino acid sequence of Pro j 2 with other allergenic plant derived profilins in the protein database was performed (Figure2). A high level of sequence identity (95%) was detected between Pro j 2 and Sal k 4 (*S. kali* pollen profilin). Moreover, the most conserved residues showed involvement in specific biological functions and structural roles (Figure2).

Expression and purification of Pro j 2

A pET-21b(+)/Pro j 2 clone was constructed and confirmed by digestion with *Not* I and *Xho* I restriction enzymes. This recombinant plasmid was expressed in *E. coli* strain BL21 (DE3)pLysS as a fusion protein with His_6-tag in the C-terminus. The rPro j 2 was present in a soluble form in the supernatant (Figure 1, lane 3), where it was further purified by Ni^{2+} affinity chromatography to yield purified protein (Figure 1, lane 4). The purified rPro j 2 was quantified by Bradford's protein assay, which showed that approximately 2.5 mg of recombinant protein had been purified from 1 liter of the bacterial expression medium. SDS-PAGE revealed that the apparent molecular weight of the fusion protein was about 18 kDa (Figure 1, lane 4).

IgE-binding analysis of rPro j 2

The levels of specific IgE resulting from exposure to the purified rPro j 2 were determined using 35 individuals patients' sera. Seventeen patients(17/35, 48.57%) had significant specific IgE levels to rPro j 2 (Table 1).

Serum samples from the patients allergic to mesquite pollen were further tested for IgE reactivity to rPro j 2by immunoblotting assays. The results showed that the recombinant form of Pro j 2 were reactive with seventeen individual sera (Figure3). These results were consistent with those obtained from specific IgE ELISA (Table 1).

In vitro inhibition assays

ELISA inhibition experiments were performed to evaluate the IgE-binding capacity of the purified rPro j 2 compared to its natural counterpart in mesquite pollen extract. ELISA inhibition results showed a dose-dependent inhibition of theIgE directed towards rPro j 2 in patients' sera positive to mesquite (Figure 4). Pre-incubation of pooled serum with 1000 µg/ml of rPro j 2 and mesquite pollen extract revealed significant inhibition (92% and 85%, respectively) of IgE binding to rPro j 2in microplate wells (Figure 4).

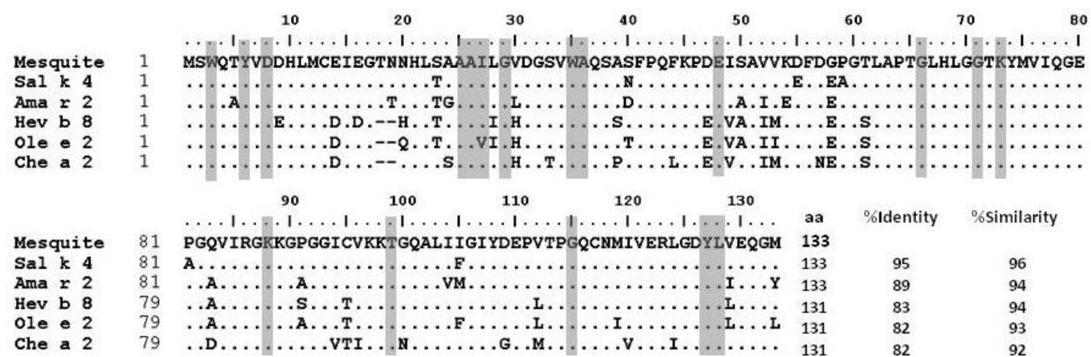

Figure 2. Comparison of the mesquite profilin amino acid sequence with allergenic profilins from other plants. The amino acid sequence identity and the similarity of Pro j 2 (KJ462467) to other members of the profilin family are indicated at the ends of the amino acid sequences. *Salsola kali* (Sal k 4, ACS34771); *Amaranthus retroflexus* (Ama r 2, ACP43298), *Hevea brasiliensis* (Hev b 8, CAA75312), *Olea europaea* (Ole e 2, A4GFC2)*Chenopodium album* (Che a 2, ACR77509). The highly conserved residues in plant-derived profilins involved in biological and structural functions (Poly-L-proline binding, fold conservation, actin binding site, and stabilized turn) are shaded in gray.

Table 1. Clinical characteristics, SPT responses and specific IgE values of patients reactive to recombinant mesquite pollen profilin.

Patients	Age (years)/sex	Clinical history[1]	Mesquite pollen extract		Recombinant mesquite profilin
			Skin test[2]	Specific IgE[3]	Specific IgE
1.	46/M	AR,RC	10	2.33	1.12
2.	39/M	AR	9	2.71	1.40
3.	26/F	AR	6	1.89	0.86
4.	43/F	AR,RC	9	1.98	0.96
5.	21/M	AR,RC	8	2.07	0.90
6.	30/M	AR	6	1.63	0.78
7.	56/M	AR,RC	7	1.97	0.95
8.	43/M	AR,RC	6	1.85	1.20
9.	32/F	AR,RC	7	1.94	0.88
10.	16/M	AR	6	2.01	1.30
11.	18/F	AR, RC	12	2.12	1.15
12.	45/F	AR	7	1.88	0.78
13.	29/F	AR,RC	10	1.93	0.85
14.	36/F	AR,RC	11	2.10	1.20
15.	32/M	AR	9	1.90	0.92
16.	19/F	AR	7	1.87	0.84
17.	25/M	AR,RC	6	1.70	0.76

[1] AR, Allergic rhinitis; RC, rhiniconjectivits. [2] The mean wheal areas are displayed in mm^2. Histamine diphosphate (10 mg/ml)-positive control; Glycerin-negative control. [3] Determined in specific ELISA as OD (optical density) at 450 nm.

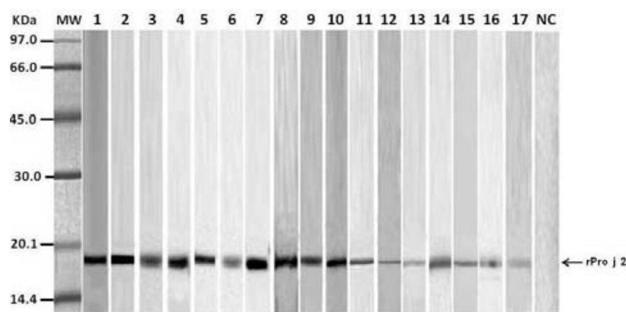

Figure 3. IgE immunoblot of purified recombinant mesquite profilin (rPro j 2) using allergic patients' sera.Lane MW, molecular weight marker (GE Healthcare, Little Chalfont, UK); lanes 1–17, probed with sera from patients with positive for rPro j 2; lane NC, negative control.

Immunoblot inhibition assays showed that pre-incubation of serum samples with rPro j 2 almost completely inhibited the ability of IgE to bind to protein band with apparent molecular weight of 15 kDa (Figure 5, lane 3). Altogether, *in vitro* inhibition assays revealed similar IgE reactivity to rPro j 2 and its natural counterpart in mesquite pollen extract. In addition, the results indicated that pre-incubation of serum samples with native crude extract of mesquite pollen completely inhibited the IgE binding to natural profiling counterparts in mesquite pollen extract and other reactive proteins (Figure5, lane 2). However, pre-incubation of the pooled serum with BSA (Figure5, lane 1) did not affect IgE-reactivity to mesquite profilin.

Discussion

Isolated profilins from various sources are characterized by high sequence identities, and for that reason, were thought to play important roles in IgE-cross reactivity in allergic patients. The purpose of this research was to clone profilin from the mesquite pollen in *E. coli* in order to evaluate its cross-reactivity with other plant-derived profilins.

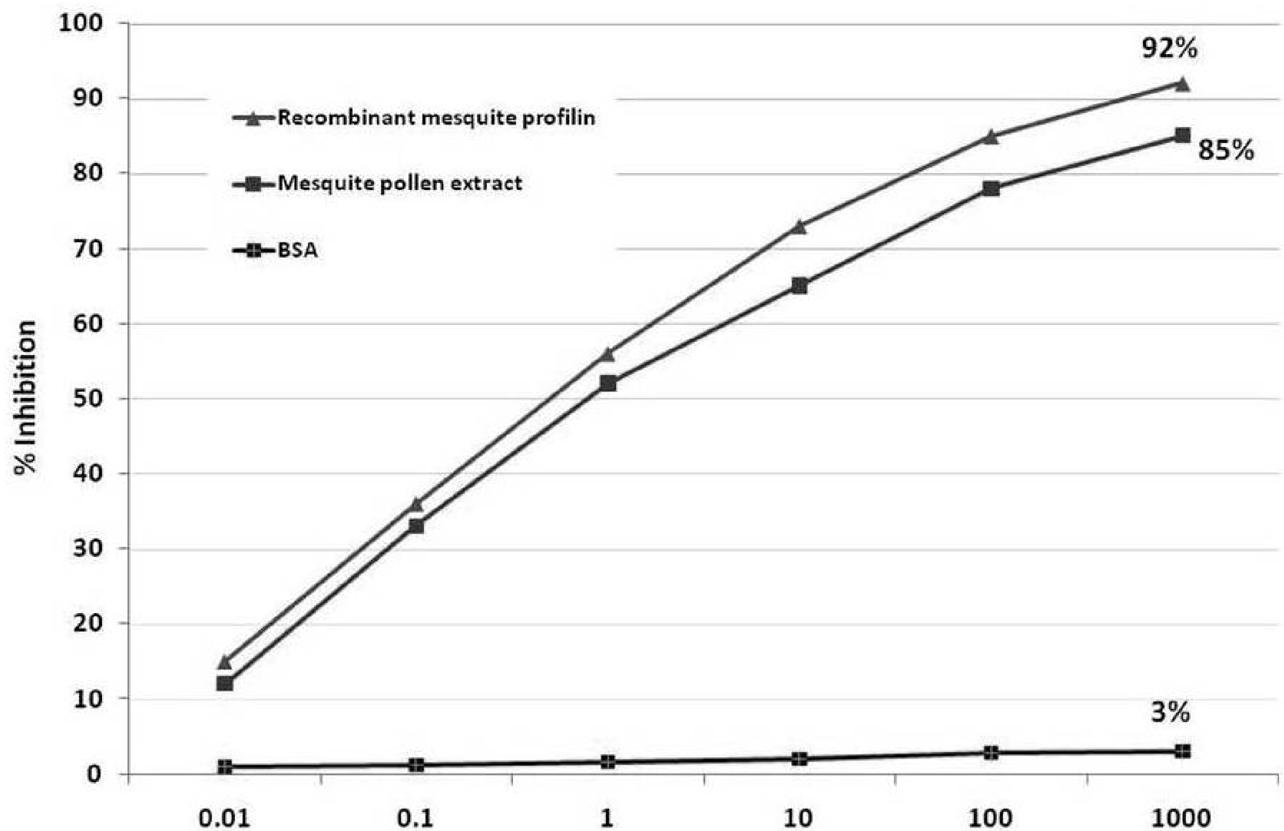

Figure 4. Inhibition of IgE-binding to rPro j 2 by ELISA using mesquite pollen extract and rPro j 2. Control experiments were performed with BSA.

In this study, profilin of mesquite pollen was cloned by PCR using degenerated primers derived from the codons of conserved amino acid sequences of various plant profilins. The open reading frame of Pro j 2 contained 399 bps encoding a 14.16 kDa protein, which correlates with the molecular characteristics of known plant profilins. Using immunoblotting, this protein was recognized in seventeen of patients allergic to the mesquite pollen extract (17/35, 48.57%). Currently described profilins are characterized by different MWs in relation to their taxonomic origin. For instance, profilins (Sal k 4, Che a 2, Ama r 2) from three members of the *Amaranthaceae/ Chenopodiaceae* families have a MW of 14.2 -14.6 kDa, while profilins from date palm pollen and oranges have a MW of 14.4 kDa and 14.0kDa, respectively[23,24] These differences may be the result of inherent variability in amino-acid residues, levels of glycosylation or sensitivity of the methods used to measure MWs.

The IgE-binding ability of the purified rPro j 2 to sera from mesquite allergic patients was evaluated in order to confirm that the recombinant protein was equivalent to its natural counterpart in crude extract. The results of immunoblotting assays for natural profilin were consistent with those obtained from rPro j 2. A complete inhibition of IgE-binding to natural mesquite profilin was also observed after pre-incubation of pooled serum with purified rPro j 2. Taken together, it seems that rPro j 2 is characterized by IgE-epitopes similar to those found in its natural counterpart.

Recently, cross-reactivity of mesquite pollen allergens with other tree species has been described.[3] In tropical areas, the importance of mesquite and most of the allergenic members of the Amaranthaceae/Chenopodiaceae families (*S. kali*, *A. retroflexus*, *C. album*) pollens have been described as causing agents of respiratory allergy.[4, 16, 25, 26] This study was carried out to assess the amino acid sequence homology of profilins from regional allergenic plants. The results showed higher identities and similarities between Pro j 2 and Sal k 4 or Ama r 2 than those of Pro j 2 and Che a 2 or Ole e 2 (Figure 2). Therefore, it appears that, although Pro j 2and Sal k 4 or Ama r 2 belong to unrelated plants, their IgE binding epitopes are highly

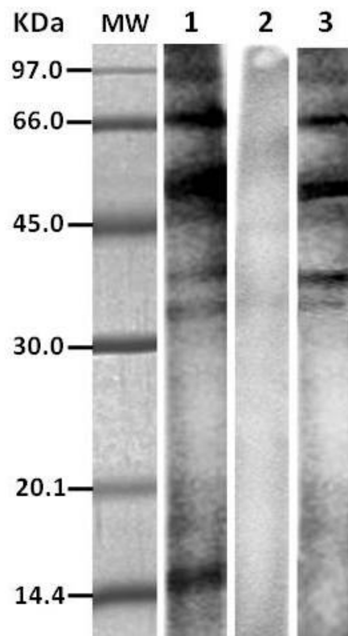

Figure5. Immunoblotting inhibition assays. Lane MW, molecular weight marker (GE Healthcare, UK); lane 1, mesquite protein strip incubated with pooled serum without inhibitor (negative control); lane 2, mesquite protein strip incubated with pooled serum containing 100μg of mesquite pollen extract as inhibitor (positive control); lane 3, mesquite protein strip incubated with pooled serum containing 100μg recombinant mesquite pollen profilin as inhibitor.

similar. Concomitant with these results, other studies have reported IgE cross-reactivities of food allergens and pollens, [27] the so called pollen-food allergy syndrome, in which there are common IgE epitopes in pollens and foods[28]. However, other studies [12, 13, 29] suggested that profilins from various plant and fruit sources exhibited differences in their IgE-binding epitopes and that cross-reactivity among these proteins were independent of their amino acid sequence homology. Radauer et al.[30] reported several conserved residues with an IgE binding potential in two predicted conformational epitopes in plant-derived profilins. In addition, to conserve residues with an IgE-binding potential, there are several other residues which are different in profilins from various sources. However, the residues playing a crucial role in the structure and biological function of the molecule are significantly conserved among unrelated phylogenic profilins.[31,32]

Recombinant mesquite profilin was successfully expressed in *E. coli* as a soluble molecule. During the process of expression and after induction with IPTG, the temperature of the culture medium was lowered to 20˚C to obtain high amount of the soluble form of the protein. This low culture temperature strategy was also suggested in previous studies.[12, 29]

In conclusion, the primary allergen in the mesquite pollen with detectable specific IgE in about 48.6% of mesquite allergic patients was shown to belong to the profiling family. The analysis of the amino acid sequences of mesquite profilin and several profilin molecules from plants belonging to unrelated taxonomic families shown cross-reactivity that may be predicted by the degree of amino acid sequence identity of potential conformational epitopes.

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