J Korean Soc Appl Biol Chem (2015) 58(3):443–451
DOI 10.1007/s13765-015-0063-5

Online ISSN 2234-344X
Print ISSN 1738-2203

ARTICLE

Immunochemical and molecular characterization of allergenic profilin (Koc s 2) from *Kochia scoparia* pollen

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Received: 2 December 2014 / Accepted: 18 March 2015 / Published online: 25 March 2015

Abstract *Kochia scoparia* pollen has been demonstrated as an important cause of pollinosis in tropical and sub-tropical regions of the world. The aim of this study was to characterize the IgE-binding protein of the Kochia pollen extract and production of recombinant form of allergenic profilin of this weed. To predict its allergenic cross-reactivity with profilins of common allergenic plants, nucleotide sequence homology of Kochia profilin was evaluated. Specific ELISA and immunoblotting assay were applied to determine the IgE-binding reactivity of 28 sera collected from patients who were sensitized to Kochia pollen. In cloning procedure, the Kochia profilin-coding sequence was inserted into PTZ57R/T vector and expressed using pET-21b(+) vector. IgE-binding competence of purified recombinant Kochia profilin (rKoc s 2) was analyzed by in vitro assays. There were several protein bands in Kochia pollen extract with molecular weights approximately ranging from 14.5 to 85 kDa. Nucleotide sequencing revealed an open reading frame of 399 bp encoding for 133 amino acid residues which belonged to the profilin family; 15 patients (15/28, 53.1 %) had significant specific IgE levels for the rKoc s 2. Immunodetection and inhibition assays indicated that the purified rKoc s 2 might be the same as that in the crude extract. Koc s 2, the first allergen from the Kochia pollen was identified as a member of the family of profilins. High degree of homology was found among amino acid sequences of Kochia profilin and several profilin molecules from unrelated plant families.

Keywords Cloning · Expression · *Kochia scoparia* · Profilin

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Introduction

Kochia scoparia (*Bassia scoparia*), a member of Amaranthaceae family, is common throughout the world such as tropical and sub-tropical regions of Asia, Africa, Europe, and America (Galan et al. 1989; Roriguez De La Cruz et al. 2011). Due to its significant resistance ability to dryness and salinity, it is found in most regions of Iran (Salehi et al. 2009). Allergy to pollens from the Amaranthaceae family (or pollinosis) has been recognized as a severe problem in desert and semi-desert areas of the world such as North, South, and West of the United States of America, Canada, Europe, Africa, some areas of Asia like Saudi Arabia, Iran, and Kuwait (Friesen et al. 2009; Tehrani et al. 2010). Previous studies, indicated that the inhalation of the Kochia pollen is one of the main causes of asthma and allergic rhinitis in Iran where the frequency of sensitization lies between 63.1 and 66.6 % (Assarehzadegan et al. 2013a, b; Bener et al. 2002; Fereidouni et al. 2009; Suliaman et al. 1997).

The recognition of allergenic components of pollens is essential for component-resolved diagnosis, the design of patient-specific immunotherapy, and the explanation of sensitization mechanisms to various allergens (Shamsbiranvand et al. 2014; Valenta and Kraft 2002).

In spite of a high rate of sensitization to the Kochia pollen in Iran and neighboring countries (Assarehzadegan et al. 2013a, b; Fereidouni et al. 2009), based on our knowledge, there are few studies about immunochemical and molecular characterization of the Kochia pollen extract. Using in vivo and in vitro assessments, therefore, the present study aimed to identify the allergenic profile of the Kochia pollen, and the cloning, expression, and purification of cDNA encoding allergenic cross-reactive proteins from the Kochia pollen. Moreover, evaluation of its nucleotide sequence homology was completed to predict allergenic cross-reactivity with the selected plant-derived profilins. The Kochia pollen profilin, as a new allergen, was designated Koc s 2 by the WHO/IUIS Allergen Nomenclature Subcommittee.

Materials and methods

Preparation of pollen and extract

As a naturally growing annual weed, the *Kochia* grows on vacant and arid lands. Samples of the polliniferous materials were collected from the field between June and September 2012, throughout the wastelands of Ahvaz city, a tropical region in southwest of Iran. Identification of the species, collection, and processing of pollen materials were done carefully by trained pollen collectors. Separation of pollen grains was performed according to previous studies (Assarehzadegan et al. 2009; Shamsbiranvand et al. 2014). Pollen materials were defatted using repeated changes of diethyl ether. Pollen was extracted as described previously (Assarehzadegan et al. 2009).

Patients and skin prick tests (SPTs)

A total of 28 respiratory allergic patients included in this study were introduced to the Immunology and Allergy Department of Ahvaz Jundishapur University of Medical Sciences (AJUMS). The patients were requested to complete a detailed questionnaire. They were considered as having a history of allergy if they reported at least one eye, nasal, or respiratory symptom to common allergens such as house dust mites, domestic animals, foods, or pollens. The patients were also evaluated by a clinical examination and a skin prick test (SPT) with common aeroallergens. Five healthy subjects who presented negative SPT and no specific IgE to the Kochia pollen extract were assigned as negative controls. Written consent was obtained from all the participants before enrollment. The human ethics committee of the university approved the study protocol. SPTs were performed by an experienced nurse under physician's supervision (Assarehzadegan et al. 2009).

Total and specific IgE enzyme-linked immunosorbent assays (ELISAs)

Total serum IgE levels were measured by means of a commercially available ELISA kit according to the manufacturer's instructions (Euroimmun, Lübeck, Germany). To measure the levels of specific IgE against the Kochia pollen in patients' sera, an indirect ELISA was developed as described earlier (Assarehzadegan et al. 2009; Shamsbiranvand et al. 2014).

SDS-PAGE and IgE-immunoblotting

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the Kochia pollen extract (60 μg) was performed according to Laemmli (1970) using 12.5 % acrylamide separation gels under reducing and non-reducing conditions. Molecular masses of bands in each gel were estimated using Image Lab Analysis Software (Bio-Rad, Hercules, CA) and then compared against known molecular weight protein markers (Amersham Low MW Calibration Kit; GE Health-care, Little Chalfont, UK).

Protein bands separated from the electrophoresis of the Kochia pollen extract were electro-transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon P, Millipore Corp., Bedford, MA, US). In brief, after transblotting, the membranes were stained, washed with PBS-T, blocked with dilution of 2 % BSA in phosphate-buffered saline (PBS, 0.15 M, pH 7.4), and incubated overnight on a shaker at 4 °C. Each strip was then incubated with a 1:5 dilution of pooled serum or individual serum of each patient with Kochia allergy or with control sera for 3 h. After washing with PBS-T, biotinylated anti-human IgE (Nordic-Mubio, Susteren, Netherlands) (1:500 v/v in PBS) was added to the blotted membrane strips and incubated for 2 h at room temperature. Washing with PBS-T, unbound antibodies were removed from the blotted membrane strips and afterwards, were incubated with 1:4000 v/v in PBS HRP-linked streptavidin (Sigma-Aldrich, St. Louis, Mo, USA) for 1 h at room temperature. After several washes in PBS-T, the strips were incubated with Supersignal West Pico Chemiluminescent Substrate Kit (Thermo Scientific, Waltham, MA, USA) for 5 min, and the proteins were then visualized by chemiluminescence using ChemiDoc XRS + system (Bio-Rad, Hercules, CA, USA).

Amplification of the Kochia profilin cDNA and determination of nucleotide sequence

Total RNA was isolated from 100 mg of the *Kochia* pollen according to Chomczynski and Sacchi method (Chomczynski and Sacchi 1987). First Strand of cDNA was

synthesized using Revert Aid^{TM} First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Degenerate primers used for cDNA amplification were designed according to consensus sequence for plant pollen profilins. The primers used were the sense 5′-ATGTCSTGGCAGAC GTAYGTHGATGA-3′ and the antisense 5′-CATGCCYTG TTCGACCAGRTARTCACC-3′. The amplified product was ligated into the PTZ57R/TTA cloning vector from InsTAclone^{TM} PCR Cloning Kit (Thermo Scientific, Waltham, MA, USA), according to the manufacturer's instruction. The ligation products were transformed into competent *Escherichia coli* TOP 10 cells (Invitrogen, Carlsbad, CA, USA) using the manufacturer's protocol. Recombinant plasmid was then purified from the gel using a Plasmid Extraction Kit (GeNet Bio, Chungnam, Korea), sequenced by the dideoxy method, and analyzed at the SeqLab Sequence laboratories (Gottingen, Germany).

Construction of prokaryotic expression plasmid carrying the Kochia profilin gene

The coding region of the Kochia pollen profilin was amplified with *Pfu* DNA polymerase (Thermo Scientific, Waltham, MA, USA) using two specific primers. The obtained sequence (GenBank accession number: KM266373) was applied to design specific primers for the *Kochia* profilin cDNA. These contained overhangs with *Not* I and *Xho* I restriction sites for direct insertion into expression plasmid pET-21b(+) (Novagen, Gibbstown, NJ, USA) as follows: the sense primer (5′-TCCGCGGCCGCAATGTCCTGG-CAGACGTATGTAGA-3′, *Not* I restriction site underlined), and the antisense primer (5′- CCCTCGAGCATGCCTTGTT CGACCAGATAGT-3′, *Xho* I restriction site underlined).

After the PCR amplification, 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 constructions were transformed into competent *E. coli* BL21 (DE3) cells (Novagen, Gibbstown, NJ, USA).

Expression and purification of recombinant rKoc s 2

A fresh clone of the recombinant plasmid pET-21b(+)/Koc s 2 profilin was inoculated into 2 ml of LB medium containing 100 μg/ml of ampicillin and then the mixture was incubated at 37 °C. Expression of the recombinant protein was induced by adding isopropyl β-D-thiogalactopyranoside (IPTG) to a concentration of 0.2 mM. After induction, in order to improve the solubility of the recombinant Kochia profilin, the cultures were incubated at 20 °C and the cells were allowed to grow for a period of 12 h. Consequently, the cells were harvested by centrifugation (3000×*g*, 15 min, 4 °C), resuspended in lysis buffer (50 mM Tris–HCl pH 6.8, 15 mM imidazole, 100 mM NaCl, 10 % Glycerol, and 0.5 % Triton X-100), and then subjected to three freeze–thaw cycles with liquid nitrogen. Supernatant of cell lysate was collected with centrifugation (8000×*g*, 10 min, 4 °C). Purification of the recombinant Kochia profilin was performed with Ni–NTA agarose (Invitrogen, Carlsbad, CA, USA) from soluble phase of lysate, following the manufacturer's instructions.

Specific and inhibition ELISAs for the rKoc s 2

In order to measure the serum specific IgE levels to the purified rKoc s 2, an indirect ELISA was developed as described above, but the wells of the ELISA microplate were coated with 6 μg/ml per well of the rKoc s 2. ELISA inhibition assays were performed as described before, but a pooled serum (1:2 v/v) from allergic patients with hypersensitivity to Kochia (Nos. 3, 9, 10 and 11) (Table 1) was pre-incubated overnight at 4 °C with either 1000,100, 10, 1, 0.1, or 0.01 μg/ml of the rKoc s 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 for the rKoc s 2

SDS-PAGE and IgE-immunoblotting for the rKoc s 2 was done as explained earlier. Using Image Lab Analysis Software (Bio-Rad, Hercules, CA, USA), the molecular masses of protein bands were estimated in comparison with known molecular weights protein markers (Amersham, GE Healthcare).

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

Results

Patients and SPTs

A total of twenty-eight patients, including 16 men and 12 women (mean age, 32.14 ± 8.71 years; age range

Table 1 Clinical characteristics, SPT responses, and specific IgE values of patients reactive to recombinant Kochia pollen profilin (rKoc s 2)

Patients	Age (years)/sex	Clinical history	Kochia pollen extract		rKoc s 2
			Skin test[a]	Specific IgE[b]	Specific IgE
1	42/M	AR, RC	12	1.60	0.90
2	28/M	AR, RC	11	1.80	1.00
3	31/M	AR	13	1.75	1.20
4	20/M	AR	8	0.98	0.95
5	23/F	AR, RC	14	1.39	0.80
6	32/F	AR	11	1.50	1.00
7	47/M	AR, RC	13	1.70	1.10
8	36/M	AR, RC	15	1.80	0.98
9	36/M	AR, RC	17	2.40	1.80
10	18/F	AR	12	1.80	1.40
11	28/F	AR, RC	15	1.90	1.20
12	23/F	AR, RC	14	1.60	0.88
13	39/M	AR, RC	13	1.54	0.85
14	42/F	AR, RC	12	1.60	1.10
15	21/M	AR	14	1.80	0.90

[a] The mean wheal areas are displayed in mm^2; Histamine diphosphate (10 mg/ml) and glycerin were used as positive and negative controls, respectively

[b] Determined in specific ELISA as OD (optical density) at 450 nm

AR Allergic rhinitis, *RC* rhinoconjunctivitis

18–47 years), suffering from respiratory allergies and seasonal rhinitis without asthma, were included in this study (Table 1). All patients had positive SPT to the Kochia extract (mean diameter of weal; 11.21 ± 2.64 mm; diameter range 7–17 mm). Moreover, pooled serum of 5 nonallergic subjects was used as a negative control.

Total and specific IgE levels

The mean total serum IgE was determined as 298.71 IU/ml (Range 123–659 IU/ml). The specific IgE levels were measured by ELISA for the Kochia pollen extract in allergic patients. All the patients showed elevated specific IgE (mean OD_{450}, 1.35 ± 0.43; range 0.80–2.40) (Table 1).

Protein and allergenic profile of the Kochia pollen extract

The reducing SDS-PAGE separation of the pollen extract showed several resolved protein bands in the Kochia extract with molecular weights ranging from approximately 14.5–85 kDa. Predominant bands had MWs of around 14.5, 39, 47, 65, 66, 80, and 85 kDa. Non-reducing SDS-PAGE of the Kochia pollen extract showed several protein bands with relatively similar molecular weights in reducing condition (Fig. 1).

IgE reactivity of the separated protein bands from the electrophoresis of the Kochia pollen extract was determined via immunoblotting assays. Specific IgE-binding fractions were probed with sera from all 28 allergic patients; the apparent MWs of each protein fraction and the prevalence of each one among all 28 allergic patients are shown in Fig. 2.

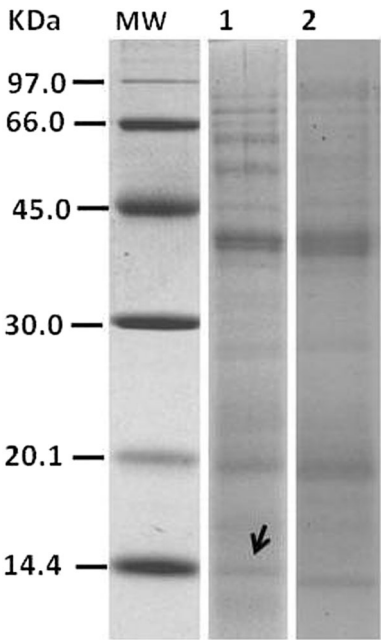

Fig. 1 SDS-PAGE of the Kochia profilin. Lane MW, molecular weight marker (GE Healthcare, Little Chalfont, UK); *lanes 1* and *2* Coomassie Brilliant Blue stained SDS-PAGE of the crude extract of the Kochia pollen in reducing and non-reducing conditions, respectively. The *arrow* shows the band corresponding to natural profilin in crude extract of Kochia pollen

The results showed that several IgE reactive bands ranged from about 14.5–85 kDa. The frequent IgE reactive bands among the patient's sera were approximately 14.5, 39, 47, 66, and 85 kDa. No band was detected when a negative control pooled serum was assayed.

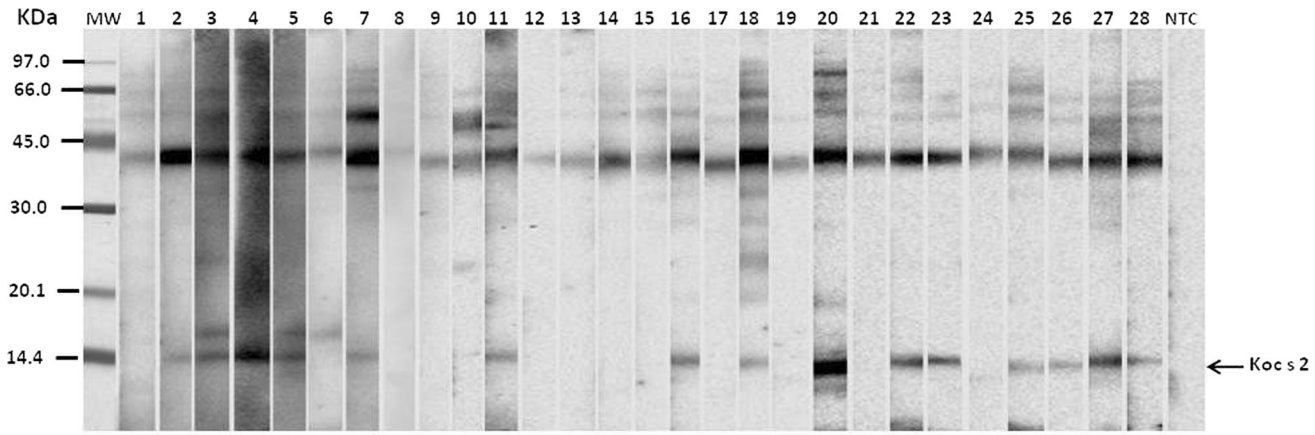

Fig. 2 Immunoblotting of the Kochia pollen extract (with reducing SDS-PAGE). Each strip was first blotted with the Kochia pollen extract, incubated with allergic patients' sera, and detected for IgE reactive protein bands. *Lane MW* low molecular weight (GE Healthcare, Little Chalfont, UK). *NTC* negative control

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                                10        20        30        40        50        60        70        80
                                |         |         |         |         |         |         |         |
Koc s 2   1  MSWQTYVDDHLMCDIEGTTNHLTGAAILGVDGSVWAQSANFPQFKPDEIAAIVKEFDEPGTLAPTGLHLGGTKYMVIQGE  80
Sal k 4   1  ............E....N....A.........................S.V......A.....................  80
Ama r 2   1  ....A........E................L.........D.............ED.....................  80
Pro j 2   1  ............E....N...SA................S.........S.V..D..G.....................  80
Ole e 2   1  ....A............--Q...A...I.H.........T......E.V...I.D.....S...................  78

                                90        100       110       120       130
                                |         |         |         |         |
Koc s 2  81  AGQVIRGKKGPGGICVKKTGQALIFGIYDEPVTPGQCNMIVERLGDYLVEQGM  133
Sal k 4  81  .....................................................  133
Ama r 2  81  P.A.......A............VM.........................I...Y  133
Pro j 2  81  P.........................I.........................  133
Ole e 2  79  P.A.......A...T................L......I.........L...Q  131
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aa	%Identity	%Similarity
133		
133	95	97
133	91	96
133	92	96
131	83	88

Fig. 3 Comparison of the Kochia profilin (Koc s 2) amino acid sequence with allergenic profilins from other plants. The amino acid sequence identity and the similarity of Koc s 2 (KM266373) to other members of the profilin family are indicated at the ends of the amino acid sequences. *Salsola kali* (Sal k 4, ACS34771.1); *Amaranthus retroflexus* (Ama r 2, ACP43298.1), *Prosopis juliflora* (Pro j 2, AHY24177.1), *Oleaeuropaea* (Ole e 2, A4GFC2.1)

Cloning and sequence analysis of the Kochia pollen profilin (Koc s 2)

The profilin of the *Kochia* pollen was cloned by PCR strategy using degenerated primers based on codons of conserved amino acid sequences from various plant profilins. The open reading frame of the *Kochia* profilin contained 399 bp encoding a 14.25 kDa protein, which correlates with the molecular characteristics of known plant profilins. The sequence analysis of the Kochia pollen profilin showed a calculated isoelectric point (pI) of 4.62. The obtained nucleotide sequence was submitted to NCBI GenBank (Accession Number: KM266373) (Fig. 3). Comparison of the deduced amino acid sequence of Koc s 2 with other allergenic plant-derived profilins in the protein database was performed. A high level of sequence identity (95 %) was detected between Kochia profilin and Sal k 4 (*S. kali* pollen profilin).

Expression and purification of the Koc s 2

A pET-21b(+)/Koc s 2 clone was constructed and confirmed by digestion with *Not* I and *Xho* I restriction enzymes. Expression of this recombinant plasmid was performed in *E. coli* strain BL21 (DE3) pLysS as a fusion protein with His_6-tag in the C-terminus. The rKoc s 2 was present in a soluble form in the supernatant, where it was further purified by Ni^{2+} affinity chromatography to yield purified protein. Quantification of the purified r Kocs 2 was done by Bradford's protein assay, which showed that approximately 2.1 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 around 18 kDa (Fig. 4a, Lane 2) because the recombinant plasmid (pET-21b(+)/Koc s 2) contained two His-tags for Ni–NTA purification.

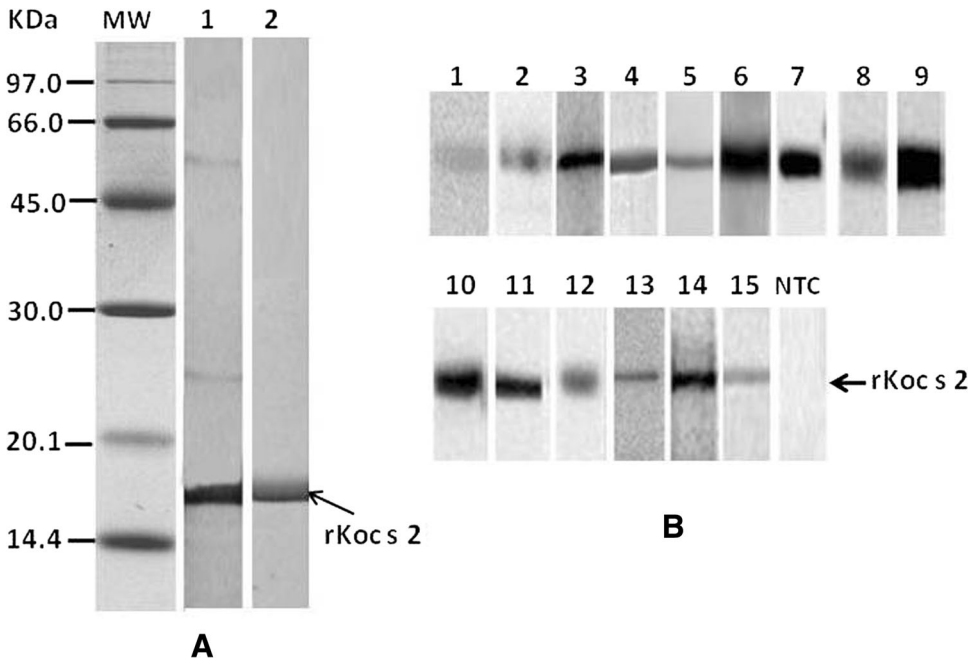

Fig. 4 SDS-PAGE and immunoreactivity of the recombinant Kochia profilin (rKoc s 2). **a** *lane 1* rKoc s 2 in soluble fraction; *lane 2* the purified rKoc s 2 (as an approximate 18-kDa recombinant protein) on 12.5 % acrylamide gel. **b** IgE immunoblot of purified rKoc s 2 using allergic patients' sera. *Lanes 1–15* probed with sera from patients positive for rKoc s 2; *lane NTC* negative control

Analysis of rKoc s 2 IgE-binding

The levels of specific IgE to the purified rKoc s 2 using 28 individual patients' sera were determined. Fifteen patients (15/28, 53.5 %) had significant specific IgE levels to rKoc s 2 (Table 1).

Serum samples from the patients who had allergy to the Kochia pollen were further tested by immunoblotting assays in order to evaluate IgE reactivity to the rKoc s 2. The results showed that the recombinant form of the Kochia profilin was reactive with 15 individual sera (Fig. 4b). 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 rKoc s 2 compared to its natural counterpart in the Kochia pollen extract. The ELISA inhibition results showed a dose-dependent inhibition of the IgE directed towards the rKoc s 2 in patients' sera positive to Kochia (Fig. 5). Pre-incubation of pooled serum with 1000 μg/ml of the rKoc s 2 and the Kochia pollen extract revealed a significant inhibition (95 and 87 %, respectively) of IgE binding to the rKoc s 2 in the microplate wells (Fig. 5).

Immunoblotting inhibition assays showed that pre-incubation of the serum samples with the rKoc s 2 almost completely inhibited the IgE binding to a protein band with an apparent molecular weight of 14.5 kDa (Fig. 6, lane 3). Altogether, in vitro inhibition assays revealed a similar IgE reactivity for rKoc s 2 and its natural counterpart in Kochia pollen extract. In addition, the results indicated that pre-incubation of the serum samples with native crude extract of the Kochia pollen almost completely inhibited the IgE binding to the natural profilin counterparts in the Kochia pollen extract and other reactive proteins (Fig. 6, lane 2). However, pre-incubation of the pooled serum with BSA did not affect its IgE reactivity to the Kochia profilin (Fig. 6, lane 1).

Discussion

Kochia, is a common weed throughout arid and semiarid areas of Iran and neighboring countries in Asia (Assarehzadegan et al. 2013a; Friesen et al. 2009; Tehrani et al. 2010). SDS-PAGE revealed several bands from the Kochia pollen extract with estimated MWs ranging from 14.5 to 85 kDa. Among those bands, four IgE-binding protein fractions with apparent MWs of 15, 39, 66, and 85 kDa were detected from the blotted proteins. In previous studies, proteins with apparent MWs of 39, 45, 66, and 85 kDa have been the most allergenic proteins in some allergenic weeds belonging to the Amaranthaceae family such as *S. kali*, *A. retroflexus*, and *C. album* (Assarehzadegan et al. 2009; Tehrani et al. 2010). There are some variations in the molecular weights of the Kochia pollen proteins of our research and those reported by previous studies (Assarehzadegan et al. 2009; Tehrani et al. 2010; Wurtzen et al. 1995). This inconsistency could be as a result of differences in pollen extracts, serum samples, concentration of

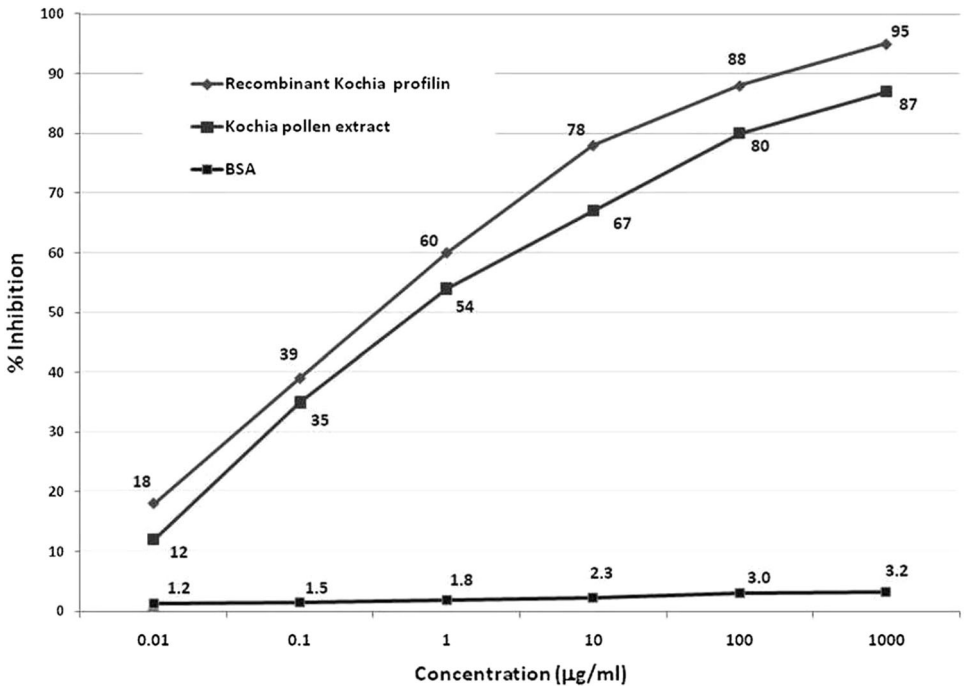

Fig. 5 ELISA inhibition with the Kochia pollen extract and rKoc s 2. Inhibition of IgE binding to rKoc s 2 by ELISA using the Kochia pollen extract and rKoc s 2. Control experiments were performed with BSA

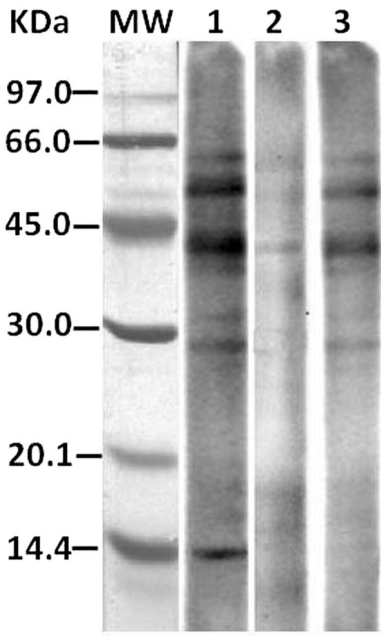

Fig. 6 Immunoblotting inhibition assays. *lane MW* molecular weight marker (GE Healthcare, UK); *lane 1* Kochia protein strip incubated with pooled serum without inhibitor (negative control); *lane 2* Kochia protein strip incubated with pooled serum containing 100 µg of Kochia pollen extract as inhibitor (positive control); *lane 3* Kochia protein strip incubated with pooled serum containing 10 µg/ml purified rKoc s 2, as inhibitor

the gels, and calculating methods of molecular weights (Dhyani et al. 2006; Killian and McMichael 2004). However, in accordance with the earlier studies, the results of the immunoblotting assays showed that in the allergenic profile of the Kochia pollen extract, the protein with apparent MW of 39 kDa is one of the major reactive proteins (Assarehzadegan et al. 2009; Tehrani et al. 2010; Wurtzen et al. 1995).

The protein profiles of the Kochia pollen extract were compared under both reducing and non-reducing conditions. The results of SDS-PAGE indicated that the pattern of the protein components migration of the Kochia crude extract did not change in reducing conditions. Thus, it was suggested that three cysteine residues of the pollen proteins might not be associated with interchain disulfide bonds.

In previous studies, it was demonstrated that some proteins in pollen extract such as methionine synthase of *S. kali* and *A. retroflexus* were partially degraded into two fragments with approximate MWs of 45 and 39 kDa (Assarehzadegan et al. 2010b; Plunkett 2008; Tehrani et al. 2010; Utley et al. 1985). Altogether, these observations suggested that as a result of proteolysis, some proteins in the Kochia pollen extract may be susceptible to degradation. Moreover, it is possible that the number or size of these degrading proteins depends on conditions of pollen extract preparation and storage. Nevertheless, additional studies are required to elucidate the patterns of degradation and the number and the size of cleavage products.

Several studies reported that proteins with apparent MWs of 39, 45, and 66 kDa are more allergenic in the pollen extracts of Kochia and the selected members of the Amaranthaceae family (Assarehzadegan et al. 2009; Carnes et al. 2003; Tehrani et al. 2010; Wurtzen et al. 1995), as

it is confirmed in the current study. Furthermore, in previous studies it was demonstrated that two bands with apparent MWs of 39 and 45 kDa were found as the most frequent IgE reactive proteins in the pollen extracts of both *A. retroflexus* and *S. kali* (Assarehzadegan et al. 2009; Carnes et al. 2003; Fereidouni et al. 2009).

Another dominant IgE-binding protein band with an estimated MW of 14.5 kDa was also detected by immunoblotting of the Kochia pollen extract. Formerly, the allergens belonging to the profilin family with apparent MWs of 14–15 kDa were found from *S. kali* (Sal k 4), *A. retroflexus* (Ama r 2), and *C. album* (Che a 2) pollens (Amini et al. 2010; Tehrani et al. 2011; Assarehzadegan et al. 2010a). The 15-kDa protein of the Kochia pollen might be homologous with the 15-kDa IgE reactive band in these plants.

Using immunoblotting, this protein was recognized in 15 individuals (15/28, 53.1 %) of patients who had allergy to the *Kochia* pollen extract. So far, different MWs for profilins of different plant sources have been reported, such as 14.2–14.6 kDa in three members of the Amaranthaceae family (Sal k 4, Che a 2, Ama r 2), 14.4 kDa in date palm pollen (Asturias et al. 1997), and 14.0 kDa in oranges (Lopez-Torrejon et al. 2005). These discrepancies may be elucidated by diversities only in some amino acid residues, levels of glycosylation, or measuring MWs methods.

To verify similarity of the recombinant protein and its natural counterpart in the crude extract, the IgE-binding ability of the purified recombinant Kochia profilin (rKoc s 2) to allergic patients' sera was evaluated. The results of immunoblotting assays for the natural profilin were consistent with those obtained from the rKoc s 2. A completed inhibition of IgE binding to the natural Kochia profilin was also obtained after pre-incubation of pooled serum with the purified rKoc s 2. Generally, it seems that rKoc s 2 comprises IgE epitopes similar to those of its natural counterpart.

The recombinant Koc s 2 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 18 °C to obtain a high amount of the soluble form of the protein. This low culture temperature strategy was also suggested by previous studies (Assarehzadegan et al. 2010a; Tehrani et al. 2011).

In tropical areas, the importance of the Kochia and the most allergenic members of the Amaranthaceae (*S. kali*, *A. retroflexus*) and Fabaceae (*P. juliflora* and *A. farnesiana*) families pollens have been described as causes of respiratory allergy (Al-Frayh et al. 1999; Assarehzadegan et al. 2013a, b; Fereidouni et al. 2009; Shamsbiranvand et al. 2014). This study was carried out to detect the amino acid sequence homology of profilins from allergenic regional plants. The results showed higher identities and similarities between the Kochia profilin and both Sal k 4 (*S. kali* profilin) and Pro j 2 (*P. juliflora* profilin) (95 and 91 %, respectively) than those of both Ama r 2 (*A. retroflexus* profilin) and Ole e 2 (*Olea europaea* profilin). Therefore, it appears that although Kochia or *S. kali* and *P. juliflora* belong to unrelated families, IgE-binding epitopes of their allergenic profilins are very similar. Concomitant with these results, other studies have reported IgE cross-reactivities of food allergens and pollens (Vieths et al. 2002; Ebner et al. 1991).

Radauer et al. (2006) 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. Nonetheless, residues playing a crucial role in the structure and biological function of the molecule are significantly conserved among unrelated phylogenic profilins (Thorn et al. 1997; Wilkes and Otto 2000).

In conclusion, the Kochia pollen is a potent allergen source with several IgE-binding components. The observations altogether suggest a close allergenic relationship between Kochia and other members of the Amaranthaceae and Fabaceae families. Finally, the first allergen from the Kochia pollen (Koc s 2) with detectable specific IgE in about 53.51 % of the Kochia allergic patients was identified as a member of the family of profilins. Analysis of the amino acid sequences of Koc s 2 and several profilin molecules from other allergenic plants also showed cross-reactivity among plant-derived profilins from unrelated families that might be predicted by the degree of amino acid sequence identity of potential conformational epitopes.

Acknowledgments This article is issued from the thesis of Miss Zarin and financial support was provided by Ahvaz Jundishapur University of Medical Sciences (Grant No. U-92211).

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