

Recombinant expression, purification and cross-reactivity of chenopod profilin: rChe a 2 as a good marker for profilin sensitization

31455

Rodrigo Barderas, Mayte Villalba and Rosalía Rodríguez*

Depto. Bioquímica y Biología Molecular, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, E-28040 Madrid, Spain

* Corresponding author
e-mail: rrg@bbm1.ucm.es

Abstract

Chenopod pollen is one of the major sources of allergens in some locations in the US, southern Europe and desert countries, and pollen profilin (Che a 2) is a major allergen. Recombinant Che a 2 (rChe a 2) has been produced in *Escherichia coli* cells with a final yield of 25 mg/l of cell culture. The expressed protein was isolated and structurally characterized by means of mass spectrometry, Edman degradation and circular dichroism. rChe a 2 displayed a molecular mass of 13 959 Da, which agrees with that of the amino acid sequence. The N-terminal amino acid sequence indicated the correct processing of the recombinant product. The immunological analysis of rChe a 2 showed IgG- and IgE-binding capabilities equivalent to those of its natural counterpart, Che a 2, isolated from the pollen. Inhibition experiments showed high cross-reactivity degrees with different allergenic sources. Inhibition degrees of >95% and >80% were obtained for chenopod profilin and, respectively, latex and pollen extracts, whereas 10–95% of inhibition was observed for different plant-derived foods. Due to its close relation to other allergenic profilins from pollens, plant-derived foods and latex, rChe a 2 could be a useful tool in clinical trials to detect profilin-allergic patients and perhaps, depending on its clinical relevance, in specific immunotherapy of these hypersensitive individuals.

Keywords: Che a 2; *Chenopodium album* pollinosis; cross-reactivity; profilin; recombinant allergen.

Introduction

Almost 25% of people worldwide suffer from type I allergy, an immunological disease that is a consequence of the production of IgE antibodies against allergens. The members of the *Chenopodiaceae* family are widespread weeds growing in temperate areas that are responsible for an increasing number of allergic cases. Allergy to chenopod (*Chenopodium album*) pollen, the weed that gives the name to this family, has been scarcely studied, prob-

ably due to its overlapping with other pollinoses (Lombardero et al., 1991). To date, three allergens have been isolated and characterized from chenopod pollen: Che a 1, Che a 2 and Che a 3 (Barderas et al., 2002, 2004). Recently, our group has reported important roles for profilin (Che a 2) and polcalcin (Che a 3) in *C. album* pollen allergy, and showed that these allergens displayed a high prevalence in patients sensitized to this pollen (Barderas et al., 2003, 2004). Although chenopod profilin was purified from the pollen, the isolation procedure yielded a very low amount of the allergen (Barderas et al., 2004).

Profilins are low molecular weight proteins found in all eukaryotic cells, where they play an important role in the regulation of the actin cytoskeleton. Their function is modulated by proline-rich peptides as well as by phosphatidylinositol phosphates. This family of well-conserved proteins, which displays amino acid sequence identities of around 75%, has been reported to contain significant allergenic members in all of the sources studied (Caballero et al., 1998). Because of their ubiquity, profilins have been described as panallergens (Valenta et al., 1992), and patients suffering from profilin allergy commonly react to a broad range of inhalant and nutritional sources. Around 20% of pollen allergic patients recognized this allergen, but it has been described as a major allergen in *C. album*, *Mercurialis annua*, *Croccus sativus*, *Phoenix dactylifera* pollens and in soybean seeds (Feo et al., 1997; Vallverdú et al., 1997; Rihs et al., 1999; Fernández et al., 2000; Barderas et al., 2004). However, profilins should be investigated by inhalation provocation tests and/or food challenge studies because they seem to have very limited clinical relevance (Wensing et al., 2002).

The *E. coli* bacterial system is widely being used to express proteins that do not need post-translational modifications. *E. coli* has been reported to be used as the host for the expression of profilins from different sources such as birch (Bet v 2), olive (Ole e 2), latex (Hev b 8), pear (Pyr c 4), cherry (Pru av 4), celery (Api g 4) and mugwort (Art v 4) (Valenta et al., 1991; Asturias et al., 1997; Rihs et al., 2000; Scheurer et al., 2001; Wopfner et al., 2002), and very recently Pru p 4 from peach (Rodríguez-Pérez et al., 2003). These recombinant profilins were obtained as soluble, immunologically active forms, which maintained their allergenic properties.

The aim of this work was to study the production of the *C. album* pollen profilin in *E. coli* as a non-fusion protein, as well as its isolation and characterization. We compared its properties with those of its natural counterpart nChe a 2. In addition, and to provide evidence on its potential use in clinical tests to determine profilin sensitization, an evaluation of its cross-reactivity with vegetable extracts from different biological sources was performed.

Results

A cDNA encoding the profilin of chenopod pollen (Che a 2) was amplified by PCR using as template pCR2.1/Chea2.1, and two degenerate oligonucleotides selected on the basis of the amino acid sequence of the protein isolated from the pollen (Barderas et al., 2004). The complete sequence of Che a 2 was subcloned into the pET11b plasmid after digestion of pCR2.1/Chea2 with *NdeI* and *EcoRI*. Intracellular expression of *E. coli* BL21 (DE3)-transformed cells with pET11b/Chea2 was evaluated by means of SDS-PAGE and Coomassie Blue staining of identical aliquots of the culture medium after 0, 0.5, 1, 2 and 4 h of induction (Figure 1). Immunoblotting and staining with an olive profilin-specific polyclonal antiserum (pAb) completed the analysis (Figure 1). A band at 14–15 kDa apparent molecular mass appeared after 1 h of induction and corresponds to rChe a 2. The recombinant allergen is thus expressed as a non-fusion protein in the soluble fraction of the harvested and disrupted cells.

Two chromatographic steps over Sephadex G-50 gel permeation columns were necessary to achieve purification of rChe a 2 (Figure 2). The procedure yielded 25 mg of soluble recombinant profilin per liter of cell culture. The purified recombinant protein was analyzed by immunoblotting for its IgG- and IgE-binding capacities against the olive profilin-specific pAb, and for a pool of chenopod-allergic sera. rChe a 2 was strongly recognized by both antisera (Figure 2).

In order to evaluate the purity of rChe a 2, an analytical RP-HPLC (Figure 3A) and a mass spectrometric analysis (Figure 3B) of the protein were performed. The sample displayed a single peak in both experiments. The molecular weight of the protein as determined by mass spectrometry (MS) was 13 959, which is close to the theoretical molecular mass deduced from the amino acid sequence (13 950 Da). The differences between both values are within the mass spectrometer deviation value of 0.1%. The N-terminal amino acid sequence of rChe a 2 was determined by Edman degradation and identified the sequence SWQTY. This is in agreement with the sequence of nChe a 2 obtained from pollen (Barderas et al., 2004) with the absence of the initial methionine because this amino acid is processed by the bacteria. These experiments established the purity of the recombinant allergen.

Accurate evidence for the folding of rChe a 2 was obtained in the spectroscopic analysis from its far-UV circular dichroism (CD) spectrum (Figure 3C). By applying the convex-constraint analysis method, the content of secondary structure of rChe a 2 was obtained: 33% α -helix, 14% β -sheet, 37% β -turn and 16% random coil. These data indicate that the expressed allergen is properly folded at the secondary structure level.

To evaluate the antigenic behavior of the expressed allergen rChe a 2 and for a comparison with that of the natural form nChe a 2, their responses against IgG and IgE antibodies were analyzed by both immunoblotting and ELISA. The recognition of purified rChe a 2 by 15 sera from chenopod-allergic patients is shown in Figure 4A. A negative control was included in the analysis. IgE-

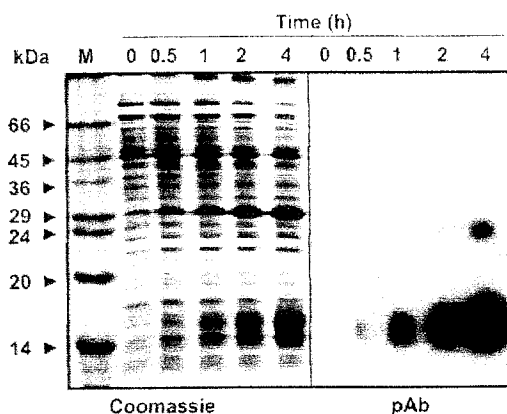


Figure 1 Time course of the expression of rChe a 2 analyzed by SDS-PAGE.

Cell lysate supernatants (0.025%) were collected at different times. Coomassie Brilliant Blue staining and immunostaining using an olive profilin-specific pAb are shown. Lane M: molecular mass markers.

binding titrations to rChe a 2 and nChe a 2 were also performed with a pool of six sera showing similar reaction with both proteins (Figure 4B).

IgG- and IgE-binding inhibition assays between rChe a 2 and nChe a 2 were performed by means of immunoblotting with anti-Ole e 2 pAb and an equivolumetric pool of six sera from individuals allergic to chenopod, respectively (Figure 5). rChe a 2 and nChe a 2 were transferred to nitrocellulose membranes after SDS-PAGE and their binding to the antibodies assayed after separate pre-incubation of the sera with the allergens as inhibitors.

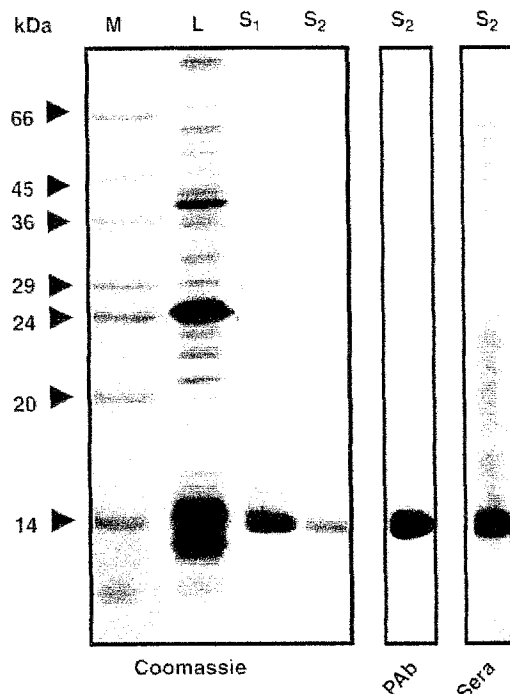


Figure 2 SDS-PAGE analysis of rChe a 2 purification steps. Pooled fractions (0.1%) of lysate (L), and the two Sephadex columns (S_1 and S_2) were analyzed. Immunostaining of purified protein (0.5 μ g) with anti-olive profilin pAb and the sera pool of chenopod pollen allergic patients was performed.

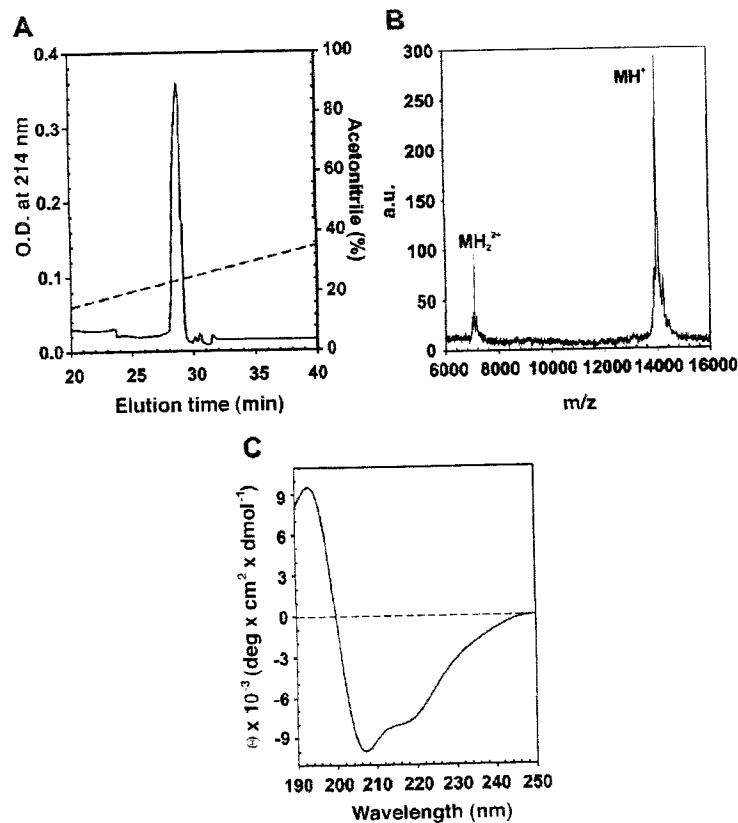


Figure 3 Purity and spectroscopic analyses of rChe a 2. (A) Reverse-phase HPLC. (B) Mass spectrum in arbitrary units (a.u.). (C) Far-UV CD spectrum.

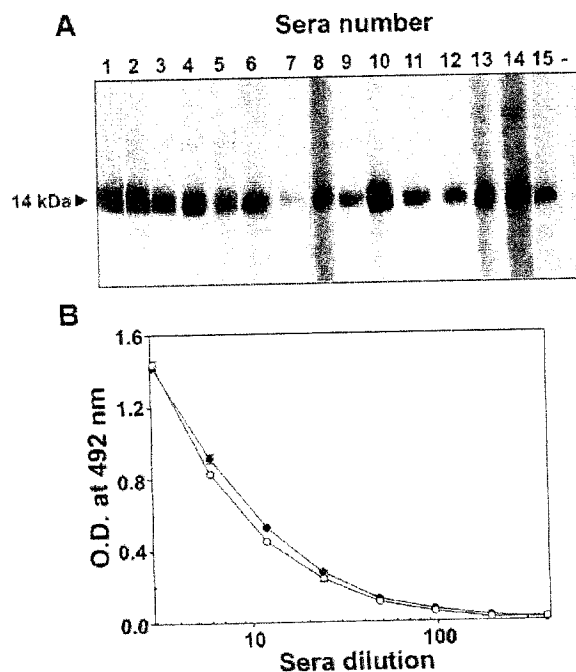


Figure 4 IgE-binding analysis of chenopod pollen profilin. (A) Immunoblotting after SDS-PAGE and blotting of rChe a 2 with 15 sera from chenopod pollen allergic patients (lanes 1–15) and a non-allergic pool of sera (lane -). (B) ELISA titration of nChe a 2 (closed circles) and rChe a 2 (open circles) with an equivolumetric pool of sera ($n=6$).

A complete inhibition was always obtained, indicating that natural and recombinant Che a 2 share all of the IgE and IgG epitopes.

The cross-reactivity of chenopod profilin was evaluated by means of inhibition ELISA experiments using several pollens, plant-derived foods and latex extracts as inhibitors and a pool of sera from patients allergic to chenopod (Table 1). Inhibition levels higher than 80% were obtained with pollen and contact allergens, whereas values ranging between 10 and 95% were achieved with

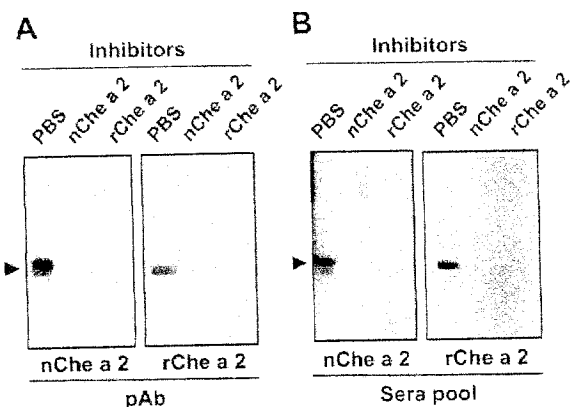


Figure 5 Immunoblotting inhibition assays with nChe a 2 and rChe a 2 allergens.

Membranes with natural or recombinant Che a 2 were incubated with pAb (A) or sera pool (B) using as inhibitors nChe a 2 (2 μ g), rChe a 2 (2 μ g) or buffer (PBS) as a negative control.

Table 1 Cross-reactivity between chenopod pollen profilin and different allergenic sources.

Pollen allergen	Profilin			
	Inhibition (%)	I (%)	S (%)	Accession no.
Chenopod	95.7±0.5	100	100	Q84V37
Russian thistle	91.2±1.3	-	-	-
Olive	96.0±0.6	78	87	O24169
Privet	95.3±0.8	-	-	-
Ash	94.5±0.4	-	-	-
Lilac	92.7±0.5	-	-	-
Birch	94.8±0.5	75	87	P25816
Plane tree	94.2±0.3	-	-	-
Cypress	87.8±0.3	-	-	-
Ragweed	88.4±0.5	-	-	-
Mugwort	86.4±0.5	74	84	Q8H2C9
Orchard grass	86.7±0.2	-	-	-
Pellitory	86.4±0.4	77	87	Q9XG85
Timothy grass	82.8±0.3	80	90	O24650

Food allergen	Profilin			
	Inhibition (%)	I (%)	S (%)	Accession no.
Potato	95.1±0.3	-	-	-
Bell pepper	90.2±0.5	80	92	Q93Y19
Tomato	90.4±0.3	82	93	Q8VWRO
Kiwi	80.1±0.3	-	-	-
Banana	67.7±3.0	82	92	Q94JN3
Peach pulp	48.5±0.7	83	93	Q8GT39
Peach peel	10.7±0.1	-	-	-

Contact allergen	Profilin			
	Inhibition (%)	I (%)	S (%)	Accession no.
Latex	95.4±0.3	83	95	Q9M7NO

IgE-binding inhibition (%) to rChe a 2-coated wells by using 200 µg (total protein) of indicated extracts as inhibitors. An equi-volumetric pool of sera (n=6) was used in the assay. The percentage of identity (I), similarity (S) and the accession number in the database banks (accession no.) of the sequenced profilins are shown.

plant-derived food allergens. Interestingly, peach peel and peach pulp showed a great difference in inhibition capacity (10 and 48%, respectively), which suggests a higher presence of profilin in pulp than in peel.

Discussion

Most of the studies on chenopod allergy have shown a high degree of cross-reactivity, not only with pollens of the *Amaranthaceae* family but also with those of taxonomically less related species (Lombardero et al., 1985; Wurtzen et al., 1995; Florido et al., 2000). This fact suggested the involvement of panallergens in the IgE reactivity of chenopod pollen. Thus, a preliminary analysis was performed with chenopod pollen protein extract to investigate the implication of profilin and the EF-hand calcium-binding protein (polcalcin) in this pollinosis (Barderas et al., 2003). One conclusion of this study was that profilin plays an important role in the allergy to chenopod. To look more deeply into these studies, we performed the purification and characterization of profilin from this pollen (Barderas et al., 2004). However, the amount of this protein in biological sources, not only from pollens but also mainly from plant foods, is quite low and a very limited quantity of the pure allergen could be obtained;

only 30 µg of nChe a 2 was obtained per gram of dried chenopod pollen (Barderas et al., 2004). A useful alternative to obtaining profilins is the heterologous expression of their recombinant forms (Valenta et al., 1991; Asturias et al., 1997; Rihs et al., 2000; Scheurer et al., 2001; Wopfner et al., 2002; Rodriguez-Pérez et al., 2003). This can provide a well-defined protein in reasonable amounts for clinical and scientific purposes. The availability of the cDNA encoding chenopod profilin made possible the production at high yield of the allergen Che a 2 in the BL21 (DE3) strain of *E. coli* cells as host. The isolation of highly pure rChe a 2 was performed by conventional chromatography steps, and the product was judged devoid of contaminants by RP-HPLC, mass spectrometry, SDS-PAGE and Edman degradation. The folding of rChe a 2 was evaluated by CD, and the secondary structure profile agrees with those obtained for profilins of olive, birch and timothy grass (Fedorov et al., 1997; Ledesma et al., 1998a). rChe a 2 was strongly reactive to anti-Ole e 2 polyclonal antiserum, and was also recognized by IgE of sera from chenopod-allergic patients in an identical way to nChe a 2. In addition, all the sera preselected by their IgE-reactivity to nChe a 2 displayed a positive response in immunoblotting to rChe a 2, and the inhibition analyses of the binding to IgG and IgE between natural and recombinant chenopod profilins resulted in a complete equivalence of the two forms of the allergen.

Considering these results, which agree with a well-folded recombinant profilin that maintains the allergenic and antigenic epitopes with respect to the natural form and the high yield in the production of rChe a 2, it looks like a suitable molecule to be used in biochemical assays and in clinical trials such as diagnosis and immunotherapy of chenopod-profilin allergic patients. Nevertheless, experiments that demonstrate the *in vivo* activity of rChe a 2 should be performed before being included in these protocols.

Profilins have been reported to be implicated in cross-reactivity between pollens, pollen-fruits and in the latex-fruit syndrome (Valenta et al., 1992; Daschner et al., 1998; Wagner et al., 2002). In addition, a role for profilin in cross-reactivity related to grass pollen allergy and peach has been suggested (Van Ree et al., 1995; Vieths et al., 2002). Recently, new pollen-fruit cross-reactivity has been reported by Callejo et al. (2002). In this study, the sera from patients with pollen allergy to chenopod showed OAS after eating banana, melon and peach. The IgE antibodies of the sera of these patients recognized allergenic bands of 10–20 kDa in the chenopod pollen extract, and a component of 14 kDa was considered as a major allergen. Melon and banana extracts inhibited the IgE binding of most of the sera to the majority of the chenopod-reactive bands, whereas the inhibition capacity of peach was less important (Callejo et al., 2002). Although profilin was not identified as the 14 kDa reactive component, it is a strong candidate to be recognized by the sera of these pollinic and fresh fruit allergic individuals. Our data support these findings. In fact, chenopod profilin has been found to have higher amino acid sequence identity with latex and food profilins than with some pollen profilins (Barderas et al., 2004). Because of

its strong cross-reactivity with olive pollen profilin and high prevalence (55%) in chenopod pollen allergic patients, it has been suggested that Che a 2 could be an important mediator in cross-reactivity (Barderas et al., 2004). In the present work, inhibition assays between rChe a 2 and pollen, and plant-related foods and contact (latex) allergens were performed. A high level of inhibition was obtained, not only for pollens (>80% of inhibition) but also with several vegetable and fruit extracts (potato, bell pepper and tomato, >90%; kiwi, >80%). Taking into account that the amino acid sequence similarity of Pru p 4 to Che a 2 is in the range of those of the chenopod profilin and tomato or bell pepper (Table 1), the lower inhibition obtained for peach (peel and pulp) extracts would be associated with the very low level of profilin in this food material (Rodríguez-Pérez et al., 2003), as has been previously suggested (Callejo et al., 2002).

Our findings suggest that rChe a 2 could be a useful tool in the diagnosis and specific immunotherapy of patients suffering from profilin sensitization.

Materials and methods

Protein extracts and chenopod profilin

Pollen from chenopod (*C. album*), Russian thistle (*Salsola kali*), olive (*Olea europaea*), privet (*Ligustrum vulgare*), ash (*Fraxinus excelsior*), lilac (*Syringa vulgaris*), birch (*Betula verrucosa*), plane tree (*Platanus acerifolia*), cypress (*Cupressus sempervirens*), ragweed (*Ambrosia trifida*), mugwort (*Artemisia vulgaris*), orchard grass (*Dactylis glomerata*), pellitory (*Parietaria judaica*) and timothy grass (*Phleum pratense*) were purchased from Allergon Pharmacia (Ängelholm, Sweden). Pollens (2%, w/v) were extracted as previously described (Barderas et al., 2002).

Protein extracts from kiwi (*Actinidia chinensis*), tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum*) and bell pepper (*Capsicum annuum*) were obtained as described (Díaz-Perales et al., 2000). Latex (*Hevea brasiliensis*) extract and peach (*Prunus persica*) peel and pulp extracts were provided by Drs. G. Salcedo (Universidad Politécnica, Madrid, Spain) and J.F. Crespo (Hospital 12 de Octubre, Madrid, Spain), respectively. Extracts were tested by Coomassie Blue staining after SDS-PAGE, and their total protein content was determined by means of the Lowry method (Lowry et al., 1951). Chenopod profilin (nChe a 2) was isolated from the pollen as described by Barderas et al. (2004).

Subcloning of Che a 2.1 and construction of expression vectors

The coding region of Che a 2.1 (Barderas et al., 2004) was amplified by PCR in order to add at the 5'- and 3'-ends of the coding sequence the restriction sites necessary for subcloning the cDNA into the expression plasmid pET11b. Che a 2.1 cDNA was amplified with the following specific oligonucleotides: a sense primer with an *NdeI* restriction site (underlined), 5'-atacat-ATGTCNTGGCARGACNTAYGT-3', and an antisense primer with an *EcoRI* restriction site (underlined), 5'-gccttaag-TTAGAGNCCYTGTYCHATNAG-3'. Degenerations at the oligonucleotide sequence were: N=any base, Y=C/T, H=T/C/A and R=G/A. The obtained PCR product was cloned into the pCR2.1 plasmid (Invitrogen, Groningen, The Netherlands), and the DNA from several clones were sequenced. Subsequently, the pCR2.1/Chea2.1 construct was digested with *NdeI* and *EcoRI* restriction enzymes and subcloned into the plasmid pET11b, obtaining the

expression vector pET11b/Chea2.1. Production of the recombinant protein as a non-fusion protein was achieved after transforming the BL21 (DE3)-competent strain of *E. coli* with pET11b/Chea2.1 vector as described (Ledesma et al., 1998b). Recombinant DNA methods were carried out by standard procedures.

Expression of Che a 2 in *E. coli* cells and purification

Overnight cultures of BL21 (DE3) containing the pET11b/Chea2.1 plasmid were diluted 10-fold with LB medium containing 0.1 mg/ml ampicillin, and grown up to an absorbance value at 600 nm of 1.0. After induction with isopropyl- β -thiogalactopyranoside (Sigma-Aldrich, Químnica, Spain) at a final concentration of 0.4 mM, cultures were maintained for 4 h at 37°C and centrifuged at 4000 g for 20 min at 4°C. The supernatant was discarded, and the pellet was re-suspended in 20 mM ammonium bicarbonate, 1 mM phenylmethylsulfonyl fluoride. A bacterial lysate was performed by three cycles of freezing and thawing at 42°C. The soluble fraction, obtained after centrifugation of this material and lyophilized, was used as the starting material for purification of the recombinant product. A gel permeation chromatography in a Sephadex G-50 column (80×3.5 cm), equilibrated in 0.2 M ammonium bicarbonate, was used as the first step of purification. Fractions containing rChe a 2 were detected with a specific polyclonal antiserum obtained against olive pollen profilin (Ole e 2). These fractions were lyophilized previously to be applied onto a Sephadex G-50 column (52×2 cm). Purified rChe a 2 was lyophilized and stored at -20°C until use.

Sera and antibodies

Fifteen sera from patients suffering from pollinosis to *C. album* used in this study were selected under the criteria described by Barderas et al. (2004) and by its proven reaction to natural Che a 2. All of the patients were also sensitized to different pollen sources such as olive, *Gramineae*, mugwort, pellitory and plane tree. A pool of sera from non-atopic subjects was used as a negative control. All patients provided informed consent. Polyclonal serum against Ole e 2 profilin was prepared by immunizing a New Zealand white rabbit over a 6-week period by weekly injections of the protein (100 μ g) in complete Freund's adjuvant (Ledesma et al., 1998a).

Analytical methods for protein characterization

RP-HPLC on a Nucleosil C₁₈ column with an acetonitrile gradient (0–60%) in 0.1% trifluoroacetic acid was used to determine the homogeneity of rChe a 2. Protein concentration of purified samples was determined by means of amino acid quantitation after hydrolysis with 5.7 N HCl. Edman degradation of the protein was performed using a 477A sequencer (PE Applied Biosystems, Foster City, USA).

Electrophoresis and immunological characterization

SDS-PAGE was performed in 15% polyacrylamide gels. Proteins were stained with Coomassie Blue or electrophoretically transferred to nitrocellulose membranes (Barderas et al., 2002). Immunodetection of 0.5 μ g of pure protein was achieved by using alternatively individual serum or a pool of sera (n=6) from patients allergic to chenopod pollen (diluted 1:10), or a rabbit polyclonal antiserum raised against olive profilin (diluted 1:10 000). For immunoblotting inhibition assays the polyclonal antiserum or the pool of sera were previously incubated with buffer (PBS), 2 μ g nChe a 2 or rChe a 2 as inhibitors. The signal

was developed by the ECL-Western blotting reagent (Amersham Biosciences, Barcelona, Spain) (Barderas et al., 2002).

IgE titration was performed by indirect ELISA in microtiter plates coated with 100 µl/well of protein (1 µg/ml) as previously reported (Barderas et al., 2002). The plates were incubated with different dilutions of a pool of sera (n=6) from chenopod allergic patients. For the ELISA inhibition assay, plates were coated with 100 µl of recombinant profilin (1 µg/ml) and then incubated with the pool of sera (diluted 1:10) pre-adsorbed with 200 µg (total protein) of different extracts as inhibitors. In both assays, the binding of human IgE was detected by mouse anti-(human IgE) antibodies diluted 1:5000 followed by horseradish peroxidase-labeled goat anti-mouse IgG (diluted 1:5000). The peroxidase reaction was measured as optical density at 492 nm (Barderas et al., 2002). Each value was calculated as the mean of two determinations. The percentage of inhibition was calculated according to the formula: inhibition (%) = $[1 - (\text{O.D. with inhibitor} / \text{O.D. without inhibitor})] \times 100$.

Spectroscopic characterization and MS analysis

The CD spectrum was obtained in the far-UV range on a Jasco J-715 spectropolarimeter (Japan Spectroscopic Co., Tokyo, Japan) as described by Ledesma et al. (1998b). The protein concentration was in the 0.20–0.25 mg/ml range. Mean residue mass ellipticities were calculated based on 107.31 as the average molecular mass/residue, obtained from the amino acid composition, and expressed in terms of Θ (degree $\times \text{cm}^2 \times \text{dmol}^{-1}$).

Purified protein was analyzed as described (Barderas et al., 2002), on a Bruker Reflex II MALDI-TOF mass spectrometer (Bruker-Franzen Analytik, Bremen, Germany) equipped with an ion source with visualization optics and a nitrogen laser (337 nm). The equipment was externally calibrated employing singly-, doubly- and triply-charged signals from cytochrome c (12 360 Da).

Acknowledgments

We thank Dr. C.Y. Pascual (Hospital Infantil La Paz, Madrid, Spain) for kindly providing the allergic sera. This work was supported by grant SAF2002-02711 from the Dirección General de Investigación (Ministerio de Ciencia y Tecnología, Spain). Rodrigo Barderas is recipient of a fellowship from the same Ministry.

References

- Asturias, J.A., Arilla, M.C., Gómez-Bayon, N., Martínez, J., Martínez, A., and Palacios, R. (1997). Cloning and expression of the panallergen profilin and the major allergen (Ole e 1) from olive tree pollen. *J. Allergy Clin. Immunol.* 100, 365–372.
- Barderas, R., Villalba, M., Lombardero, M., and Rodríguez, R. (2002). Identification and characterization of Che a 1 allergen from *Chenopodium album* pollen. *Int. Arch. Allergy Immunol.* 127, 47–54.
- Barderas, R., Villalba, M., Batanero, E., Pascual, C.Y., and Rodríguez, R. (2003). The role of profilin and polcalcin in chenopod pollen allergy. *J. Allergy Clin. Immunol.* 111, 1132–1133.
- Barderas, R., Villalba, M., Pascual, C.Y., Batanero, E., and Rodríguez, R. (2004). Profilin (Che a 2) and polcalcin (Che a 3) are relevant allergens of *Chenopodium album* pollen. Isolation, amino acid sequences and immunological properties. *J. Allergy Clin. Immunol.* 113, 1192–1198.
- Caballero, T., and Martín-Esteban, M. (1998). Association between pollen hypersensitivity and edible vegetable allergy: a review. *J. Invest. Allergol. Clin. Immunol.* 8, 6–16.
- Callejo, A., Sanchis, M.E., Armentia, A., Moneoa, I., and Fernández, A. (2002). A new pollen-fruit cross-reactivity. *Allergy* 57, 1088–1089.
- Daschner, A., Crespo, J.F., and Pascual, C.Y. (1998). Specific IgE to recombinant vegetal panallergen (rBet v 2) and fruit allergy in pollinic patients. *Allergy* 53, 614–618.
- Díaz-Perales, A., Lombardero, M., Sánchez-Monge, R., García-Sellés, F.J., Pernas, M., Fernández-Rivas, M., Barber, D., and Salcedo, G. (2000). Lipid-transfer proteins as potential plant panallergens: cross-reactivity among proteins of *Artemisia* pollen, *Castanea* nut and *Rosaceae* fruits, with different IgE-binding capacities. *Clin. Exp. Allergy* 30, 1403–1410.
- Fedorov, A.A., Ball, T., Valenta, R., and Almo, S.C. (1997). X-ray crystal structures of birch pollen profilin and Phl p 2. *Int. Arch. Allergy Immunol.* 113, 109–113.
- Feo, F., Martínez, J., Martínez, A., Galindo, P.A., Cruz, A., García, R., Guerra, F., and Palacios, R. (1997). Occupational allergy in saffron workers. *Allergy* 52, 633–641.
- Fernández, J., Asturias, J.A., and Martínez, A. (2000). Biological standardization and allergen characterization of date palm pollen (*Phoenix dactylifera*). *Allergy* 55, 140.
- Florido, J.F., Quirarte, J., Saénz De San Pedro, B., and Bartolomé, B. (2000). Cross-reactivity between *Olea europaea* and *Chenopodium album* pollens. *Allergy* 55, 158.
- Ledesma, A., Rodríguez, R., and Villalba, M. (1998a). Olive-pollen profilin. Molecular and immunologic properties. *Allergy* 53, 520–526.
- Ledesma, A., Villalba, M., Batanero, E., and Rodríguez, R. (1998b). Molecular cloning and expression of active Ole e 3, a major allergen from olive-tree pollen and member of a novel family of Ca^{2+} -binding proteins (polcalcins) involved in allergy. *Eur. J. Biochem.* 258, 454–459.
- Lombardero, M., Duffort, O., Selles, J.G., Hernández, J., and Carreira, J. (1985). Cross-reactivity among *Chenopodiaceae* and *Amaranthaceae*. *Ann. Allergy* 54, 430–436.
- Lombardero, M., Duffort, O., and Carreira, J. (1991). Allergenic significance of chenopod pollen. In: *Allergenic pollen and pollinosis in Europe*, G. D'Amato, F.T.M. Speksma and S. Bonini, eds. (Oxford, UK: Blackwell Scientific Publishers), pp.128–131.
- Lowry, O., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- Rihs, H.P., Chen, Z., Rozynek, P., Baur, X., Lundberg, M., and Cremer, R. (2000). PCR-based cloning, isolation, and IgE-binding properties of recombinant latex profilin (rHev b 8). *Allergy* 55, 712–717.
- Rihs, H.P., Chen, Z., Rueff, F., Petersen, A., Rozynek, P., Heilmann, H., and Baur, X. (1999). IgE binding of the recombinant allergen soybean profilin (rGly m 3) is mediated by conformational epitopes. *J. Allergy Clin. Immunol.* 104, 1293–1301.
- Rodríguez-Pérez, R., Fernández-Rivas, M., González-Mancebo, E., Sánchez-Monge, R., Díaz-Perales, A., and Salcedo, G. (2003). Peach profilin: cloning, heterologous expression and cross-reactivity with Bet v 2. *Allergy* 58, 635–640.
- Scheurer, S., Wangorsch, A., Nerkamp, J., Skov, P.S., Ballmer-Weber, B., Wuthrich, B. et al. (2001). Cross-reactivity within the profilin panallergen family investigated by comparison of recombinant profilins from pear (Pyr c 4), cherry (Pru av 4) and celery (Api g 4) with birch pollen profilin Bet v 2. *J. Chromatogr. B. Biomed. Sci. Appl.* 756, 315–325.
- Valenta, R., Dúchene, M., Vrtala, S., Birkner, T., Ebner, C., Hirschwehr, R., Breitenbach, M., Rumpold, H., Scheiner, O., and Kraft, D. (1991). Recombinant allergens for immunoblot diagnosis of tree-pollen allergy. *J. Allergy Clin. Immunol.* 88, 889–894.
- Valenta, R., Duchene, M., Ebner, C., Valent, P., Sillaber, C., Deviller, P., Ferreira, F., Tejkl, M., Edelman, H., and Kraft, D. (1992). Profilins constitute a novel family of functional plant pan-allergens. *J. Exp. Med.* 175, 377–385.
- Valverdú, A., García-Ortega, P., Martínez, J., Martínez, A., Esteban, M.I., de Molina, M., Fernández, J., Bartolomé, B., and Palacios, R. (1997). *Mercurialis annua*: characterization of

- main allergens and cross-reactivity with other species. *Int. Arch. Allergy Immunol.* 112, 356–364.
- Van Ree, R., Fernández-Rivas, M., Cuevas, M., Van Wijngaarden, M., and Aalberse, R.C. (1995). Pollen-related allergy to peach and apple: an important role for profilin. *J. Allergy Clin. Immunol.* 95, 726–734.
- Vieths, S., Scheurer, S., and Ballmer-Weber, B. (2002). Current understanding of cross-reactivity of food allergens and pollen. *Ann. NY Acad. Sci.* 964, 47–68.
- Wagner, S., and Breiteneder, H. (2002). The latex-fruit syndrome. *Biochem. Soc. Trans.* 30, 935–940.
- Wensing, M., Akkerdaas, J.H., van Leeuwen, W.A., Stapel, S.O., Bruijnzeel-Koomen, C.A., Aalberse, R.C., Bast, B.J., Knulst, A.C., and van Ree, R. (2002). IgE to Bet v 1 and profilin: cross-reactivity patterns and clinical relevance. *J. Allergy Clin. Immunol.* 110, 435–442.
- Wopfner, N., Willeroidee, M., Hebenstreit, D., van Ree, R., Aalbers, M., Briza, P., Thalhamer, J., Ebner, C., Richter, K., and Ferreira, F. (2002). Molecular and immunological characterization of profilin from mugwort pollen. *Biol. Chem.* 383, 1779–1789.
- Wurtzen, P.A., Nelson, H.S., Lowenstein, H., and Ipsen, H. (1995). Characterization of Chenopodiales (*Amaranthus retroflexus*, *Chenopodium album*, *Kochia scoparia*, *Salsola pestifer*) pollen allergens. *Allergy* 50, 489–497.

Received March 26, 2004; accepted June 11, 2004