

Allergenic reactivity of the melon profilin Cuc m 2 and its identification as major allergen

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Summary

Background: Melon allergy is commonly associated with oral allergy syndrome (OAS) and with hypersensitivity to pollens and other plant foods. No melon allergen responsible for these clinical characteristics has yet been isolated, although profilin has been proposed as a potential target.

Objective: To isolate natural and recombinant melon profilin, to evaluate its *in vivo* and *in vitro* reactivity, and to analyse its behaviour in simulated gastric fluid (SGF) and heat treatments.

Methods: A pool or individual sera from 23 patients, and an additional group of 10 patients, all of them with melon allergy, were analysed by *in vitro* and *in vivo* tests, respectively. Natural melon profilin (nCuc m 2) and its recombinant counterpart (rCuc m 2) were isolated by poly-L-proline affinity chromatography, and characterized by N-terminal amino acid sequencing, matrix-assisted laser desorption/ionization analysis, DNA sequencing of cDNAs encoding rCuc m 2, and immunodetection with anti-profilin antibodies. *In vitro* analysis included IgE immunodetection, specific IgE determination, ELISA-inhibition assays, and histamine release (HR) tests. *In vivo* activity of nCuc m 2 was established by skin prick testing (SPT). The effect of SGF and heat treatment on rCuc m 2 was followed by immunodetection, ELISA inhibition, and HR assays.

Results: Both purified forms of melon profilin were recognized by rabbit anti-profilin antibodies and IgE of sera from allergic patients, and showed molecular sizes typical of the profilin family. nCuc m 2 had a blocked N-terminus, whereas rCuc m 2 rendered the expected N-terminal amino acid sequence, its full protein sequence being highly similar (98–71% identity) to those of profilins from plant foods and pollens. The natural allergen displayed a slightly higher IgE-binding capacity than its recombinant counterpart. Specific IgE to nCuc m 2 and rCuc m 2 was found in 100% and 78% of the 23 individual sera analysed, respectively. nCuc m 2 evoked positive SPT responses in all (10/10) patients tested, and rCuc m 2 induced HR in two out of three sera assayed. SGF treatment readily inactivated rCuc m 2, as shown by its loss of recognition by anti-profilin antibodies, lack of IgE binding, and inability to induce HR. In contrast, heat treatment did not affect the IgE-binding capacity of rCuc m 2.

Conclusions: Profilin is highly prevalent in melon-allergic patients, and promptly inactivated by SGF, as expected for an allergen mainly linked to OAS.

Keywords cross-reactions, melon, oral allergy syndrome, profilin, simulated gastric fluid digestibility
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Introduction

Melon (*Cucumis melo*) is a relevant plant food involved in the induction of IgE-mediated-allergic reactions by ingestion in several ample geographic areas, such as Central and Mediterranean Europe and United States [1–3]. Melon allergy was initially described in association with ragweed pollinosis and oropharyngeal symptoms [3]. Subsequently, several works have confirmed the link between hypersensitivity to

melon and allergy to different pollens, even in ragweed-free areas [4]. Oral allergy syndrome (OAS) is recognized as the most common clinical manifestation of melon allergy [1, 2, 5–7]. Moreover, cross-reactions of melon with other fruits [1, 4, 7, 8] and with latex [9] have been also reported. The existence of melon allergens potentially associated with OAS and with a wide spectrum of cross-reactivity could explain the main characteristics of allergy to this fruit.

Three melon allergens have been described so far. Cuc m 1 (cucumisin) is a 67 kDa subtilisin-like protease [10] and Cuc m 3 is a 16 kDa pathogenesis-related protein belonging to the PR-1 family [11]. No plant allergen homologous to Cuc m 3 has been detected until now, and although proteases related to Cuc m 1 have been located in several plant species, their

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allergenic activity remains to be demonstrated. Furthermore, the clinical significance of both allergens is unknown at present. By contrast, a third allergen, Cuc m 2, corresponding to a 13 kDa IgE-binding component of melon pulp [12], seems to be an attractive candidate to be involved in OAS and cross-reactions. Specific IgE to this component has been found in up to 70% of sera from pollinic patients with allergy to melon showing OAS. Interestingly, the 13 kDa protein band is unaffected by crude human saliva, but readily loses its IgE-binding properties with simulated gastric fluid (SGF) treatment [12]. Unfortunately, the putative 13 kDa allergen was not purified and characterized, but preliminary evidence strongly suggested that it was melon profilin.

Plant profilins are 14 kDa (131–134 amino acid residues) cytosolic proteins involved in regulating the actin cytoskeleton (see reference [13] for a review). They constitute a highly conserved (amino acid sequence identities over 70% between most pairs of members) panallergen family, which is present in almost every plant source investigated. Profilins seem to be one of the main responsible agents of cross-sensitization between plant foods and pollens, but prevalence of IgE to profilins is usually detected in only 20–30% of plant food-allergic and pollinic patients. Furthermore, the clinical relevance of profilin sensitization is still controversial [14]. A constrain to search the actual allergenic reactivity of profilins from fruits and vegetables is that virtually no natural forms are available, their recombinant counterparts being used in most studies [14, 15]. However, recombinant profilins, such as rBet v 2, seem to be poor reagents to evaluate profilin sensitization [14].

We report the isolation and characterization of natural and recombinant melon profilin Cuc m 2, as well as the high prevalence of specific IgE to this allergen, its strong *in vivo* reactivity, and its inactivation by SGF treatment.

Materials and methods

Patients and sera

Sera from 23 adult patients with melon allergy selected at the Food Allergy Unit of the Hospital Universitario 12 de Octubre (Madrid) were tested in this study. A pool of sera from five atopic subjects with allergy to house dust mites but not to plant foods or pollens was used as negative control. Pooled or individual sera from this group of melon-allergic patients were used for IgE immunodetection, direct ELISA and ELISA inhibition, and basophil histamine release (HR) assays. In addition, a second group of 10 patients also allergic to melon and two control healthy subjects were selected for skin prick test (SPT) with purified Cuc m 2. The criteria for selection were (1) a convincing clinical history of allergic reactions after melon ingestion, (2) a positive prick by prick test response to fresh melon, and (3) a specific IgE level to this fruit greater than 0.35 kU/L, as determined by using the Pharmacia CAP system FEIA (Pharmacia Diagnostic, Uppsala, Sweden). All selected patients showed multiple pollen reactivities, mainly to grasses and olive pollen. SPT was performed with pollen samples of 19 different sources: *Olea europaea*, *Fraxinus excelsior*, *Ulmus americana*, *Quercus ilex*, *Populus* spp., *Platanus acerifolia*, *Betula verrucosa*,

Dactylis glomerata, *Secale cereale*, *Phleum pratense*, *Poa pratensis*, *Alopecurus* spp., *Lolium perenne*, *Taraxacum officinale*, *Artemisia* spp., *Plantago lanceolata*, *Chenopodium album*, *Cupressus arizonica*, and *C. sempervirens*. Written informed consent was obtained from patients and control subjects.

Preparation of melon extract

Melon pulp (*C. melo* var. Piel de Sapo) was defatted with cold acetone (2 × 1:5 (w/v), 1 h, 4 °C) and, after drying, extracted with phosphate-buffered saline (PBS) buffer (0.1 M sodium phosphate, pH 7.0, and 0.15 M NaCl; 1 × 1:5 (w/v), 1 h, 4 °C). After centrifugation (12100 × *g*, 30 min, 4 °C), the supernatant was dialysed (cut-off point 3.5 kDa) against H₂O and freeze dried. Protein concentration was quantified according to the method of Bradford [16].

Isolation and characterization of melon profilin

Melon pulp extract was resuspended in H₂O (1:10 w/v), centrifuged (12100 × *g*, 20 min, 4 °C), and the supernatant subjected to affinity chromatography on a poly-L-proline-Sepharose 4B column prepared as previously described [17]. Elution was performed with 10 volumes of PBS buffer, followed by 4 volumes of 2 M urea in PBS buffer, and finally profilin was eluted with 6 M urea in PBS buffer. Profilin containing fractions, tested with anti-profilin antibodies, were pooled, dialysed against H₂O, and freeze dried. The same protocol was followed to isolate recombinant profilin from supernatant of culture media from *Escherichia coli* cells expressing the allergen. Purified allergens were quantified by means of the commercial bichinchoninic acid test (Pierce, Cheshire, UK).

SDS-PAGE was carried out according to Laemmli [18] on Bio-Rad Miniprotein II System (Bio-Rad Laboratories, Hercules, CA, USA) gels (15% polyacrylamide).

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry analysis and N-terminal amino acid sequencing were performed by standard methods with a Biflex-III spectrometer with delayed extraction (Bruker-Franzen Analytik, Bremen, Germany) and an Applied Biosystems 477A gas-phase sequencer (Foster City, CA, USA), respectively.

Isolation of cDNAs encoding melon profilin Cuc m 2 and heterologous expression of the allergen

Total RNA from melon pulp was obtained by the method of Chang et al. [19]. Reverse transcription was performed using the Marathon cDNA Amplification Kit (Clontech, Palo Alto, CA, USA). The cDNA produced was amplified by PCR using the 5'-Adaptor Primer (AP1 primer of the Marathon kit) and the oligonucleotide 3' primer 5'-TTACAGACCCTGCTC-GATAAG-3', designed from the 3'-coding region of profilin cDNAs previously reported. The amplification was carried out with Ampli-Taq Gold DNA polymerase (Perkin-Elmer, Branchburg, NY, USA) as recommended by the manufacturer. The PCR programme was 10 min denaturation at 95 °C, followed by 35 cycles each of 30 s denaturation at 95 °C, 30 s annealing at 70 °C, and 1 min and 30 s extension at 72 °C, and final extension for 7 min at 72 °C. The PCR products were cloned into pGEM T-Easy vector (Promega,

Madison, WI, USA), and these constructs were used to transform *E. coli* XL1-Blue cells. The DNAs from five clones were sequenced using the ABI PRISM Dye Terminator Kit and an ABI 3100 sequencer (Perkin-Elmer Biosystem, Warrington, UK).

To produce rCuc m 2 in *E. coli*, the coding region of the Cuc m 2 cDNA was amplified by PCR with a sense primer 5'-CATATGTCGTGGCAGGCGTACGTC-3' (*Nde*I site underlined) and an antisense primer 5'-CTGCAGTTACAGACCCTGCTC-3' (*Pst*I site underlined). The PCR products were purified and digested with *Nde*I/*Pst*I restriction enzymes, and inserted into the same sites of the pRSET plasmid (Invitrogen, Paisley, UK) to transform *E. coli* XL1-Blue cells. The DNA from four clones was sequenced to confirm the construction in pRSET plasmid. The pRSET/Cuc m 2 DNA was then used to transform *E. coli* BL21(DE3) cells. The cultures were grown in LB (100 µg/mL carbenicillin) up to an OD₆₀₀ of 0.6, and then 0.6 mM IPTG was added to the culture. The incubation continued for another 3 h. Cells were harvested by centrifugation, the pellet resuspended in 50 mM Tris-HCl, pH 7.5, and 125 mM NaCl, and finally disrupted by sonication. Insoluble material was removed by centrifugation, and the supernatant dialysed against H₂O and freeze dried was used as starting material to isolate rCuc m 2 as described above.

Immunodetection assays

Samples (20 µg of protein from *E. coli* supernatant containing pRSET or pRSET/Cuc m 2 DNA, 20 µg of crude melon extracts, and 1 µg of purified profilins, nCuc m 2 and rCuc m 2) were fractionated by SDS-PAGE and then electrotransferred onto polyvinylidene difluoride (PVDF) membranes. After washing and blocking, membranes were incubated overnight with a serum pool from patients with melon allergy or with control sera (1:5 dilution), and then treated with mouse anti-human IgE mAb HE-2 ascitic fluid [20] (1:3000 dilution) for 2 h. After washing, a rabbit anti-mouse IgE peroxidase-conjugate antibody (DAKO A/S, Glostrup, Denmark; 1:5000 dilution) was added for 1 h. Detection of IgE-binding components was carried out by enhanced chemiluminescence, following the manufacturer's instructions (ECL-Amersham Biosciences, Little Chalfont, UK).

Alternatively, replica gels were electrotransferred onto PVDF membranes. After washing and blocking, membranes were incubated with a rabbit polyclonal antibody against pear profilin Pyr c 4 (kindly provided by Dr Scheurer, Paul-Ehrlich Institute, Laugen, Germany; 1:10000 dilution). Membranes were then treated with alkaline phosphatase-conjugated anti-rabbit IgG (Sigma, St Louis, MO, USA; 1:500 dilution), and finally revealed with a 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium solution.

Specific IgE binding to Cuc m 2 and ELISA-inhibition assays

Specific IgE binding to nCuc m 2, rCuc m 2, and melon PBS extract was determined by using a method previously described [21]. The purified allergens (2 µg/mL) or the melon extract (20 µg/mL) was used as solid phase, and 23 individual sera from patients allergic to melon (1:3 dilution) were tested. BSA (1% in PBS buffer) was assayed as negative control, as

well as a serum pool from control subjects (OD below 0.05 units when tested towards nCuc m 2). All tests were performed in triplicate.

ELISA-inhibition assays were carried out by the same method. Comparison of the IgE-binding inhibition capacity of nCuc m 2 and rCuc m 2 was assayed with 2 µg/mL of each purified allergen as solid phase and the pool of sera from melon-allergic patients (1:3 dilution) pre-incubated with serial dilutions (range 10–0.02 µg/mL, 3 h, 25 °C) of the natural or recombinant allergen as inhibitor. The effect of SGF and heat treatment on rCuc m 2 was tested by using PBS melon extract (20 µg/mL) as solid phase and the same diluted pool of sera pre-incubated with serial dilutions (20–0.001 µg/mL) of untreated rCuc m 2, SGF-digested rCuc m 2 and 100 °C heated rCuc m 2 as inhibitors for 3 h at 25 °C. Melon PBS extract and nCuc m 2 (10 and 20 µg/mL), and SGF and BSA were used as positive and negative controls, respectively. All tests were performed in triplicate.

SPTs

SPTs were performed with isolated nCuc m 2 (50 µg/mL) according to standard procedures. Histamine phosphate (10 mg/mL) and PBS buffer/glycerol solution were used as positive and negative controls, respectively. A mean weal diameter of 3 mm or greater (15 min after puncture) compared with that produced by the negative control was considered a positive response.

HR assays

Heparinized venous blood (2 mL) was drawn from three patients with allergy to melon (nos. 1, 2 and 4 of Fig. 4), selected for presenting specific IgE to nCuc m 2, as well as from one non-allergic subject as negative control. Heparinized whole blood (100 µL of 1:7 buffer diluted) was incubated with 50 µL serial 10-fold dilutions (1 pg–10 ng) of either rabbit anti-human IgE (specific for ε-chains; DAKO A/S), the appropriate allergen preparation, or a buffer control (spontaneous HR) at 37 °C for 30 min. Samples were immediately centrifuged at 4 °C, and histamine was measured in the supernatant by competitive enzyme immunoassay (Histamine kit; Immunetech Pharmaceuticals, San Diego, CA, USA). Total histamine content was determined after cell lysis with H₂O. The test was considered positive when the amount of histamine released by the allergen (minus spontaneous release with buffer alone) exceeded 10% of the total histamine content.

SGF and heat treatment of rCuc m 2

SGF treatment of purified rCuc m 2, and peach LTP rPru p 3 used as control, was performed essentially as described by Astwood et al. [22]. The purified allergens (0.5 µg/µL) were dissolved in 50 µL of pre-warmed 100 mM HCl, pH 1.2, and 30 mM NaCl, or in the same solution (150 µL) containing 0.32% (w/v) of pepsin A (Sigma). Digestion was carried out with continuous shaking for 30 min at 37 °C. Aliquots of this solution (50 µL) were withdrawn at 1 and 30 min. Heat treatment was performed with the purified allergens dissolved in H₂O (0.5 µg/µL) during 15 min at 100 °C. SGF-digested and heat-treated samples were promptly frozen in liquid nitrogen,

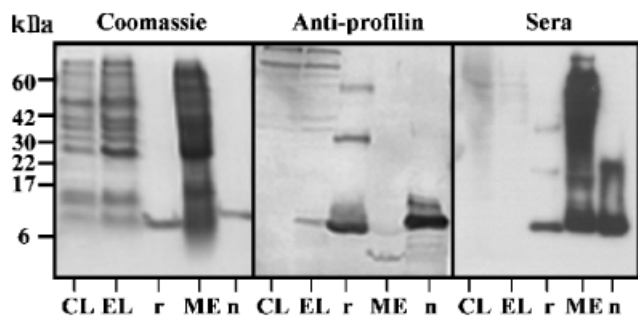


Fig 1. Protein staining (Coomassie) and immunodetection with anti-pear profilin Pyr c 4 rabbit polyclonal antibodies (Anti-profilin) or IgE of a pool of sera from melon-allergic patients of the following SDS-PAGE separated samples: control (CL) and transformed to express rCuc m 2 (EL) *Escherichia coli* lysate supernatants, purified rCuc m 2 (r), melon phosphate-buffered saline extract (ME), and purified nCuc m 2 (n).

freeze dried, and separated by SDS-PAGE on 10–20% polyacrylamide Tricine pre-cast gels (Invitrogen, DeSchelp, the Netherlands). After being electrotransferred onto PVDF membranes, samples were immunodetected with anti-Pyr c 4 or anti-Pru p 3 rabbit polyclonal antibodies, or with a pool of sera from patients with melon allergy, as described above.

Results

Fractionation of the melon pulp PBS extract by affinity chromatography on a poly-L-proline-Sepharose column led to isolation of a putative profilin, which behaved as a single protein band of around 13 kDa in SDS-PAGE and strongly bound IgE of sera from melon-allergic patients (Fig. 1). Besides its affinity to poly-proline, the purified allergen (herein nCuc m 2) was recognized by anti-profilin antibodies (Fig. 1) and showed a molecular mass of 13 997 Da as determined by MALDI analysis, thus supporting its identification as plant profilin. N-terminal amino acid sequencing

of two different batches of the purified allergen, either freeze dried or as membrane-transferred band after SDS-PAGE, rendered no sequence, indicating that nCuc m 2 had a blocked N-terminus. A very low amount of the natural profilin (approximately 0.16% of the total protein) was recovered from the melon extract.

To extend the characterization of Cuc m 2 and to produce larger quantities of the allergen, cDNAs coding melon profilin was isolated by PCR amplification and subsequently used to transform *E. coli* cells for the heterologous expression of rCuc m 2. Sequencing of five independent cDNAs showed an identical 393 bp open-reading frame (see accession number AJ565931, EMBL database, for the nucleotide sequence) coding for a 131 amino acid protein (Fig. 2), with calculated molecular weight of 14 048 Da and isoelectric point of 4.67. The deduced amino acid sequence for Cuc m 2 was very close to those previously established for allergenic profilins (Fig. 2) from fruits, such as peach Pru p 4 (98% identity), latex, and pollens (74% and 71% identity with birch Bet v 2 and ragweed profilin, respectively). Surprisingly, only 76% of sequence identity was found with Persian melon profilin isoforms (accession numbers AY293285-87) recently reported. Recombinant Cuc m 2 (2.2 mg/L) was produced in *E. coli* cells transformed with pRSET/Cuc m 2 cDNA and purified from the cell lysates by the same protocol used to isolate nCuc m 2 (Fig. 1). Proper expression and purification of rCuc m 2 was confirmed by its behaviour as a single band in SDS-PAGE, and its recognition by anti-profilin antibodies and IgE of sera from melon-allergic patients (Fig. 1). Furthermore, its determined N-terminal amino acid sequence, SWQAYVDDHL, fully matched that expected, without the N-terminal methionine usually processed in *E. coli* (Fig. 2). The same is true for its experimentally observed molecular mass of 13 968 Da by MALDI analysis. However, rCuc m 2 displayed a lower IgE-binding capacity than nCuc m 2, both in ELISA-inhibition assays (Fig. 3) and in IgE immunodetection (Fig. 1, compare lines r and n in sera panel).

Cuc m 2	MSWQAYVDDHLCDIDGNRLT--AAAI LQDGSVWSQSATFFAFRLIEIAAILKDFDQPG 58
Pru p 4	KP..... 58
Hev b 8	...T.....H.....I·H.....SS·Q·KSD·V·VM···E·· 58
Che a 2	...T.....E·H·S·S···H···A·PS·Q·KP·VS·M··NE·· 58
Bet v 2	...T·E·····QASNSL·S·V·H···A·SS·Q·KPQ·TG·M··EE·· 60
Ambrosia	E····E·TGHHLTS··LGH···A·SN·Q·KP·MKG·ITE··A·· 60
Cuc m 2	TLAPTGLFLGGTKYMIQGEAGAVIRGKKGSGGITVKKTNQALIIGIYDEPLTPGQCNI 118
Pru p 4	 118
Hev b 8	S····H····P····G···· 118
Che a 2	S····H····P·D····P·V·I····G·M····V 118
Bet v 2	H····H···I····I···G··VF··E·V····V 120
Ambrosia	····M·IA·A···L··Q····A··CI··G··VM····VA····V 120
Identity (%)	
Cuc m 2	VERLGDYLIQGL 131 -
Pru p 4	 131 98
Hev b 8	LD·· 131 84
Che a 2	···I···V···M 131 75
Bet v 2	···L···ID·· 133 74
Ambrosia	···L···ID··M 133 71

Fig 2. Alignment of the complete amino acid sequence deduced from cDNA clones encoding Cuc m 2 and those of plant profilins from fruit (peach Pru p 4; accession no. AJ491881), latex (Hev b 8; accession no. AJ243325) and pollens (*Chenopodium album* Che a 2; accession no. AY082337, *Betula verrucosa* Bet v 2; accession no. M65179 and *Ambrosia artemisifolia*; accession no. AY268427). Amino acid sequence variations and identities (%) with respect to Cuc m 2 are shown.

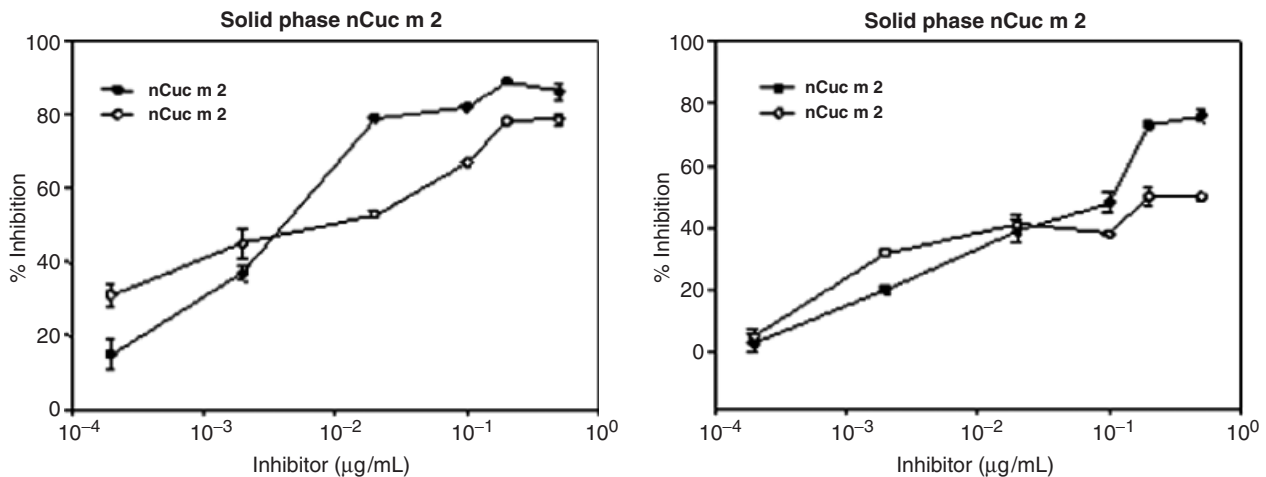


Fig 3. ELISA-inhibition with nCuc m 2 or rCuc m 2 in the solid phase and as inhibitors. Means ($n=3$) and SEs (bars) are represented.

Specific IgE to natural and recombinant Cuc m 2 (2 µg/mL in solid phase) and to PBS extract from melon pulp (20 µg/mL) was assessed in 23 individual sera from patients with melon allergy (Fig. 4). Significant IgE levels to nCuc m 2, rCuc m 2, and PBS melon extract were detected in 23 (100%), 18 (78%), and 16 (64%) of the sera, respectively.

The allergenic activity of Cuc m 2 was further tested by two different methods. Firstly, nCuc m 2 was assayed *in vivo* by SPT, obtaining 10 out of 10 (100%) positive responses in melon-allergic patients, but no reaction in two healthy control subjects. Secondly, nCuc m 2 and rCuc m 2 were assayed in basophil HR tests (Table 1), inducing HR in two out of three sera from patients sensitized to melon with significant IgE to the natural allergen.

To evaluate the stability of melon profilin, rCuc m 2 was subjected to SGF and heat treatments (Fig. 5). SDS-PAGE separation followed by immunodetection indicated that the allergen was extensively digested by SGF within 1 min of treatment (not shown), completely losing its capacity

to bind anti-profilin antibodies and IgE from melon-allergic patients after 30 min (compare lanes + and - of Cuc m 2 in Fig. 5). In contrast, heat treatment (15 min at 100 °C) did not affect either the electrophoretic mobility or the binding to IgE of rCuc m 2. Peach LTP rPru p 3, used as positive control, was resistant to both SGF digestion and heating, as previously described [23]. These results were further supported by ELISA-inhibition assays (Fig. 6). Untreated or heat-treated rCuc m 2 reached an inhibition of 42% and 50%, respectively, of the IgE binding to PBS melon extract, whereas the maximum inhibition value obtained by SGF-digested rCuc m 2 was 18%, very similar to that found (16%) for SGF tested as negative control (not shown). The PBS melon extract and nCuc m 2 inhibited 78% and 57% in the same assays. The inactivation of rCuc m 2 by SGF treatment was also confirmed by basophil HR tests (Table 1). On the contrary, the heated allergen induced significant levels of HR in one of the two sera that responded to untreated rCuc m 2.

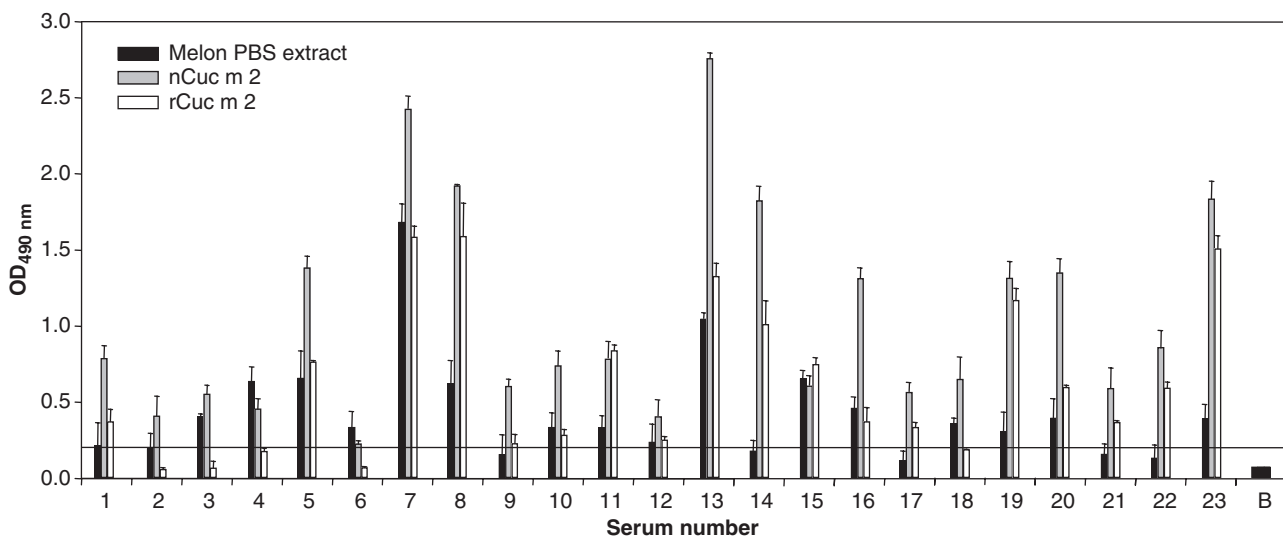


Fig 4. Specific IgE to nCuc m 2, rCuc m 2, and phosphate-buffered saline (PBS) melon pulp extract of 23 individual sera from patients with melon allergy. BSA (B) was tested as negative control. Means ($n=3$) and SEs (bars) are represented. Significant specific IgE levels were considered positive when $OD \geq 3 \times OD(0.075)$ of the mean ($n=5$) obtained for the negative control.

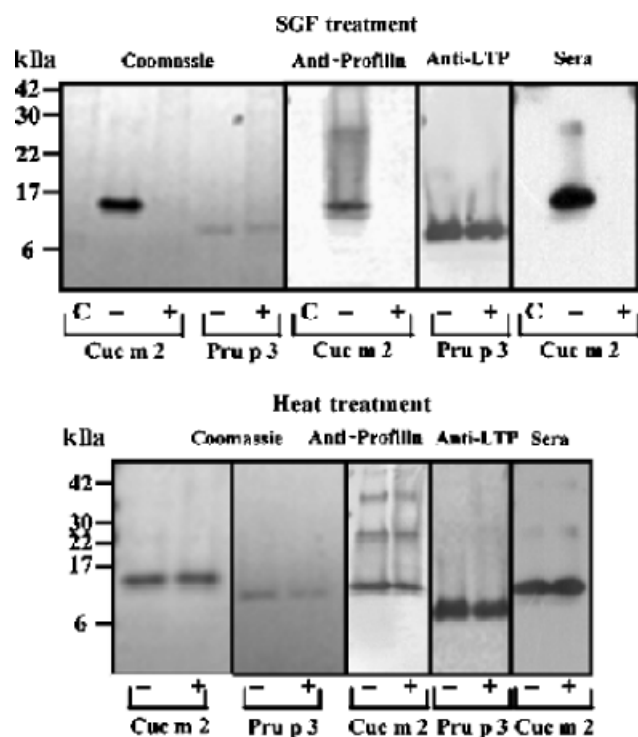


Fig 5. Protein staining (Coomassie) and immunodetection with anti-Pyr c 4 (Anti-profilin) or anti-Pru p 3 (Anti-LTP) rabbit polyclonal antibodies, or IgE from melon-allergic patients (Sera) of untreated (–) or treated (+) samples of purified rCuc m 2 and rPru p 3. SGF (C) was included as negative control.

Discussion

Several studies on melon allergy were carried out with different series of patients since its first description in 1970 [3]. Melon allergy is usually associated with hypersensitivity to pollens and other plant foods, OAS being its most common clinical manifestation [1, 2, 4–8]. In fact, only a low proportion of allergic subjects to melon show monosensitization or severe systemic reactions. The group of patients analysed in this work fully conforms to this clinical pattern as previously described for the majority of them [1]. The scenario of allergy to melon summarized above suggests that

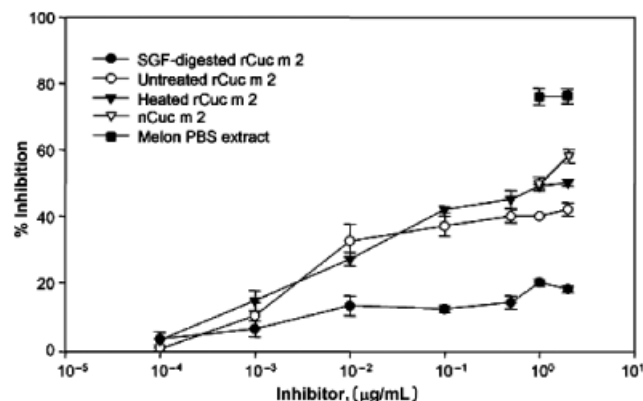


Fig 6. ELISA-inhibition with melon pulp extract in the solid phase and the same extract, nCuc m 2, rCuc m 2, and SGF- and heat-treated rCuc m 2 as inhibitors. The assays were performed with a serum pool from melon-allergic patients. Means ($n = 3$) and SEs (bars) are represented.

Table 1. Histamine release induced by nCuc m 2 and rCuc m 2, and by heat-treated (HT) and simulated gastric fluid (SGF)-digested rCuc m 2 using basophils of patients allergic to melon

Serum no.	Maximum histamine release (%)				
	nCuc m 2	rCuc m 2	HT rCuc m 2	SGF rCuc m 2	SGF
1	– (0.6)	– (0.4)	– (0.2)	– (1.2)	– (1.0)
2	11.1	14.5	– (5.5)	– (0.6)	– (0.9)
3	43.4	36.7	49.9	– (3.1)	– (1.2)
Control	– (1.4)	– (0.4)	– (0.1)	– (0.4)	– (0.3)

Values are means ($n = 2$). SGF and serum from a non-allergic subject (control) were used as negative controls. Histamine release $>10\%$ were considered positive. Serum 1, 2 and 3 correspond to nos. 4, 1 and 2 of Fig. 4.

the types of allergens mainly involved are probably cross-reactive molecules widely distributed in allergenic sources of plant origin and susceptible to inactivation by digestive proteases. In agreement with this hypothesis, Rodríguez-Pérez et al. [12] have recently detected a 13 kDa band in melon extracts, which bound IgE of 71% of sera from allergic subjects, and was readily digested with SGF. Unfortunately, the reactive component could not be purified and characterized, but indirect evidence suggested its profilin nature. Plant profilins are labile panallergens [13], thus fitting the properties expected for putative melon allergens causing co-sensitization to plant foods and pollens and local oropharyngeal symptoms. However, their clinical relevance is still not clear [14], and their prevalence (determined by the presence of specific IgE) in most pollen and plant food-allergic population is scarce (around 10–30%) [13]. Moreover, the low profilin content in extracts from most fruit and vegetables, together with its tendency to aggregate, have usually prevented the isolation of natural forms, and, consequently, their use in diagnostic trials. The recombinant profilins commonly assayed seem to be, at least in some cases, weaker reactive tools [14].

We have purified both the natural and recombinant melon profilin Cuc m 2 to clarify the role of this panallergen in sensitization to melon fruit. The proper isolation and identification of both Cuc m 2 forms were supported by their single bands shown after SDS-PAGE, affinity to poly-L-proline, molecular sizes determined by MALDI analysis, recognition by rabbit anti-profilin antibodies, and reciprocal inhibition in ELISA tests. nCuc m 2 seemed to be blocked at the N-terminus, whereas the N-terminal amino acid sequence experimentally determined for rCuc m 2 fully matched (except the processed N-terminal Met) that deduced from its corresponding cDNAs. The complete amino acid sequence deduced for the recombinant allergen allowed to demonstrate its close similarity to other allergenic plant profilins from foods (only two differences in 131 residues with peach rPru p 4) and pollens (74% sequence identity with birch Bet v 2). Furthermore, a high sequence identity (66%) between Cuc m 2 and Bet v 2 was also found in those regions of Bet v 2 (residues 1–52 and 106–132) involved in IgE binding [24]. A higher (approximately 20%) IgE binding to nCuc m 2 than to rCuc m 2 was observed in IgE immunodetection and ELISA-inhibition assays using a pool of sera from melon-allergic patients. The differential IgE binding was supported by the specific IgE levels to each allergen form detected in the 23

individual sera forming the pool, being higher for nCuc m 2 in 21 of them. Thus, natural melon profilin seems to be a better diagnostic tool, at least *in vitro*, than its recombinant counterpart, as previously reported for profilins from other plant sources [13, 14].

A very high prevalence, 100% for nCuc m 2 and 78% for rCuc m 2, was found *in vitro* for melon profilin, according to the specific IgE determinations in individual sera. This result was further confirmed *in vivo* by testing the natural form in 10 patients with melon hypersensitivity, obtaining positive SPT responses in all of them. Moreover, both nCuc m 2 and rCuc m 2 induced HR from basophils in two out of three blood samples of patients allergic to melon. Interestingly, Asero et al. [25] have recently uncovered that, among 35 plant foods analysed, a clinical history of allergy to melon and/or watermelon is the best clinical marker of sensitization to profilin in patients with allergy to plant-derived foods.

Isolated rCuc m 2 was readily inactivated by SGF treatment, thus indicating its susceptibility to gastrointestinal digestion. The inactivation was demonstrated by the lack of recognition by anti-profilin antibodies, the loss of IgE binding both in immunodetection and ELISA-inhibition assays, and the failure to induce basophil HR from sera reactive with untreated rCuc m 2 in the same assays. Preliminary reported data on a putative profilin band in crude melon extract [12], and SGF digestion of cherry profilin rPru av 4 [26] are in line with these results. In contrast, heat treatment of rCuc m 2 did not affect either its IgE-binding capacity, as indicated by the similar responses displayed by untreated and heated samples in IgE immunodetection and ELISA-inhibition assays, or its biological activity in HR test. Resistance to heat treatment of other profilins from plant foods has been derived from studies using crude extracts, as far as we know [13].

The isolation of natural and recombinant forms of melon profilin has allowed to evaluate the potential relevance of the allergen in patients with melon allergy. Moreover, the data presented here constitute a reasonable base to explain the restriction of mild symptoms to the oropharyngeal cavity and the co-sensitization to pollens and other plant foods, commonly shown by melon-allergic patients. Whether or not sensitization to profilin can occur by the oral route in the same populations, as well as the role played by other melon allergens recently reported [10, 11], particularly in patients with severe and systemic symptoms, remains to be investigated.

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