

Mango profilin: cloning, expression and cross-reactivity with birch pollen profilin Bet v 2

Juanjuan Song · Hongyun Zhang · Zhigang Liu · Pixin Ran

Received: 28 December 2006 / Accepted: 26 February 2007
© Springer Science+Business Media B.V. 2007

Abstract Mango can cause severe anaphylactic reactions. Profilin has been assumed partly responsible for the cross-reactivity between mango fruit and other allergens but has not been finally clarified. In this study, two isoforms of mango fruits profilin were amplified by RT-PCR and 3'RACE from total RNA. Each mango profilin cDNA includes an open reading frame coding for 131 amino acids. The deduced amino acid sequence of the corresponding protein show high identity with other allergenic profilins. Expression of the recombinant mango profilin was carried out in *Escherichia coli* BL21(DE3) using vector PET28a and the purification of the recombinant protein was performed via affinity chromatography with Ni⁺ coupled to sepharose. IgE reactivity of recombinant mango profilin was investigated by immunoblot and 8 of 18 mango-allergic patients tested presented specific IgE-antibodies to recombinant mango profilin. IgE-inhibition and ELISA inhibition experiments were performed to analyze mango profilin cross-reactivity with profilins from birch pollen and high cross-reactivities have been found.

Keywords Cross-reactivity · Mango · Profilin · Recombinant allergen

Introduction

The mango, scientific name *Mangifera indica* L. is one of the most popular tropical fruits and the mango tree is cultivated worldwide. Like other species in Anacardiaceae family, such as pistachio and cashew, mango can cause severe anaphylactic reactions [1–3]. Vargas et al. [4] studied 71 subjects and found that the prevalence of cutaneous sensitive to mango were 66%. A French study showed that in a group of 580 patients with proved food allergy, 6% were sensitized to mango fruits [5].

Up to now, very little information is available for the mango fruit allergen. Recently, cross-reactivity between mango fruits and other food and pollen have been reported, and the so-called 'celery-mugwort-spice syndrome' and 'latex-fruits syndrome' with allergy to mango fruits have also been described [4, 6, 7]. Paschke et al. [8] have studied the cross-reactivity between mango fruit, birch pollen, mugwort pollen, celery and carrot and found that the cross-reactivity was partly caused by the major birch pollen allergen Bet v 1, which is a 17-kDa glycoprotein. They also assumed that profilin maybe partly responsible for the cross-reactivity between mango fruit and other allergens, but the role of profilin in the cross-reactivity of mango fruit has not been finally clarified.

Profilin are 14–17 kDa proteins which are found in all eukaryotic cells. They were first described by Carlsson et al. [9] as actin-binding proteins in calf spleen, which inhibited the growth of actin filaments in vitro. Allergologists' attention was drawn to profilin in 1991 when a highly cross-reaction minor birch pollen allergen Bet v 2 was identified as a profilin [10]. Since then, profilins were identified as allergen of many fruits, vegetables, foods, as well as pollen. And studies have turned out that profilins are highly conserved with an identities range between 70

J. J. Song · H. Y. Zhang · Z. G. Liu (✉)
College of life science, Shenzhen University, Shenzhen 518060,
China
e-mail: liuzhg@126.com

J. J. Song · P. X. Ran
Guangzhou Medical College, Guangzhou 510182, China

and 80% among known allergenic plant profilins [11]. Its ubiquity and high sequence conservation characteristics have led to the term of 'pan-allergen' for this protein.

In the present study, the amino acid sequence of two isoforms of mango fruit profilin were obtained from their corresponding cDNA and one of those isoforms was expressed in *E.coli*. The recombinant protein was purified by Ni²⁺ affinity chromatography and its IgE-binding capabilities were studied by means of immunoblot experiments. Moreover, the cross reactivity between recombinant mango profilin and Bet v 2 was investigated by immunoblot inhibition and enzyme-linked immunosorbent assay (ELISA) inhibition experiments.

Materials and methods

Recombinant allergens and protein extracts

Recombinant birch pollen profilin rBet v 2 was obtained from Biomay (Linz, Austria). Protein extracts from mango fresh fruit were prepared by a low temperature method as described by Pastorello [12] with minor modifications. In brief, pieces of mango fruit were ground in a mortar filled with liquid nitrogen. The obtained powder was homogenized in pre-chilled acetone and stored overnight at -20°C. The precipitates were washed twice with acetone, filtered and lyophilized. Protein extracts were obtained by incubating the powder with phosphate buffer (0.15 M NaCl/0.01 M NaH₂PO₄) at 4°C. After centrifugation, the supernate was collected, filtered and freeze-dried. The lyophilized extracts were stored at -80°C.

Cloning of mango fruit profilin cDNA

Total RNA was isolated from mango fruits using the 'RNeasy Plant RNA Mini Kit' (Qiagen) according to the manufacturer's instructions. The reverse transcription was carried out with 2 µg total RNA using AMV First-strand cDNA synthesis Kit (BBI), the reaction was performed at 42°C for 60 min followed by heated denaturation at 95°C for 5 min.

The cDNA obtained was amplified by polymerase chain reaction (PCR) using the degenerated oligonucleotide primers (5'-primer 5'-ATGTCGTGGCAGGCGTACGT-3', 3' primer 5'-CAA CAA(C/T)CATGTTGCA(T/C)TTGTC C-3'). The primers were designed from the known sequence of different plant profilins. The amplification was performed with Ex-Taq DNA polymerase (Takara) as recommended by the manufacturer. The PCR program was carried out as follows: after denaturation at 94°C for 3 min, 9 touchdown cycles were performed at 94°C for 1 min, 65°C for 45 s and 72°C for 1 min, with the annealing

temperature decreasing by 1°C per cycle, followed by 25 cycles of each 1 min denaturation at 94°C, 45 s annealing at 58°C, and 1 min extension at 72°C, and final extension for 10 min at 72°C. After purification, the PCR products were ligated into pMD18-T vector (Takara), the constructed plasmids were transformed into *E.coli* Top10. The positive clones were sequenced by Sangon Biotechnology company (Shanghai, China) using the ABI 337 sequencer.

3'-RACE (Rapid amplification of 3' cDNA end) and sequence analysis

The clones obtained from the degenerated primers did not cover the full coding region. For amplification of the unknown 3'-end of the mango profilin, the 3'-RACE method was used according to the manufacturer's instructions (3'-full RACE Core set, TaKaRa). First-strand cDNA synthesis was primed with an Oligo dT-3sites adaptor primer enclosed in the 3'RACE system. The first strand cDNA was amplified by PCR using a gene-specific primer and the 3-sites adaptor primer supplied by the 3'-RACE system. The PCR conditions were similar to those mentioned anterior with a minor modification that the annealing temperature was at 58°C during the all reaction. PCR products were also cloned into the pMD18-T vector and transformed into *E.coli* Top10, the positive clones were sequenced. The full-length cDNAs were obtained by the overlapping of the truncated gene and its 3'-end. The identities of the mango profilin cDNAs and their deduced amino acid sequences with profilin from other plant sources were analyzed using the Blast program in the NCBI website (<http://www.ncbi.nlm.nih.gov>) and the alignment of amino acid sequences were generated by CLUSTAL W (1.83) multiple sequence alignment program.

Expression and purification of the recombinant mango profilin

In order to ligated into the PET-28a expression vector, *Nde* I and *Xho* I restriction sites were introduced at the 5'-end and 3'-end of the mango profilin cDNA, respectively, by PCR using primers as MpNde (5'-CATATGTCGTGGC AGGCGTAC-3') and MpXho (5'-ATCCTCGAGCTA AAGACCTTGTTCACA-3'). Initial cloning was done in *E.coli* strain Top10. Positive clones were selected first by colony PCR with the MpNde and MpXho primers and then by digested with the *Nde* I and *Xho* I restriction enzymes and sequencing. The target plasmids were transformed into *E.coli* BL21 (DE3) cells for expression.

The cells were grown in LB-medium supplemented with 50 µg/ml kanamycin to an absorbance of 0.6 at a wavelength of 600 nm. Expression of mango profilin was

induced by adding isopropyl- β -thiogalactopyranoside (IPTG) to LB medium at a final concentration of 0.1 mM and the incubation was continued overnight at 37°C. Cells were harvested by centrifugation and resuspended in 50 mM Tris-HCl, 100 mM NaCl, pH7.5. After three cycles of freeze-thawing, cells were sonicated to obtain the lysate, insoluble material was removed by centrifugation. Finally recombinant protein was purified by affinity chromatography using the Ni²⁺ as solid phase. The supernatant of the lysate was applied to the column followed by washing with 40 mM imidazole and elution with 200 mM imidazole. The elution products were dialyzed three times against 0.02 M Tris-HCl buffer and then freeze-dried.

Measurement of the quantities of proteins

The quantities of all proteins used in this paper were measured by Bradford assay method [13, 14]. In brief, a set of standards is created from bovine serum albumin (BSA) whose concentration is known. The absorb values (absorbance at 595 nm) obtained for the standard are then used to construct a standard curve, and the concentration of the samples were determined by comparing the absorb values with the standard curve.

Patients' sera

Sere samples used in this study were taken from 18 birch-pollen-allergic patients with a positive case history of immediate type reaction to mango fruits. All patients reported additional allergies to other pollen such as amaranth, pigweed, pine and typical pollen related foods such as lychee, orange or peanut, and only patients' sera with a positive skin prick testing result to mango (>7 mm²) were used in the study. Sera from nonallergic subjects served as negative control. All sera were kindly supplied by Shenzhen people's hospital and all samples were stored at -80°C.

IgE-immunoblotting and immunoblot inhibition

Protein from *E.coli* lysate and purified recombinant profilin were analyzed by SDS-PAGE. Proteins were visualized by Coomassie Brilliant Blue R250 staining or immunoblotting. For immunoblot analysis recombinant profilin (0.5 μ g/cm slot) were electrically transferred onto the nitrocellulose membranes and the membrane were blocked overnight with 2% bovine serum albumin solution (BSA) at 4°C, then allergic patients' sera (diluted 1:5) were added to each membrane strip for 2 h at 37°C. The biotin-labelled goat anti-human IgE antibody (KPL) were used as the secondary antibody, followed by incubation with the streptavidin-conjugated-HRP at 37°C for 2 h. The blotting

bands were finally visualized with diaminobenzidine (DAB) chromogenic substrate solution.

For inhibition of IgE-binding to recombinant mango profilin, a pooled patient serum was preincubated with 100 μ g mango extracts, or 15 μ g of recombinant birch profilin rBet v 2, or recombinant mango profilin, respectively. The pre-absorbed serum pool was then used for immunoblot experiments. The PBS was used as control.

ELISA and ELISA Inhibition

For ELISA, 96 well Maxisorb plates were coated with 0.5 μ g recombinant mango profilin in PBS overnight at 4°C. Plates were blocked in PBS/2% bovine serum albumin for 2 h at 37°C and were washed three times with PBS/0.05% Tween-20, and then the plates were incubated with patients' sera (diluted 1:5) overnight at 4°C. The biotin-labelled goat anti-human IgE antibody was used as the secondary antibody, followed by incubation with the streptavidin-conjugated-HRP at 37°C for 1 h. Each incubation step was followed by a three-times washing with PBS/0.05% Tween-20. After visualization of the enzymatic activity with the AB liquid as substrate, the reaction was stopped by the addition of 100 μ l 2 M H₂SO₄ and absorption was measured at a wavelength of 450 nm.

For ELISA inhibition, sera were preincubated for 2 h at 37°C with 0.1, 1, 10 and 100 μ g/ml of the inhibition protein (mango extracts, rBet v 2, and mango profilin) before overnight incubation on the allergen-coated plates. Then the inhibition experiment was performed as ELISA experiments described above.

Results

Cloning of the mango profilin cDNA and sequence analysis

By amplification of cDNA from first strand synthesis products with degenerated oligonucleotide primers based on the known profilin sequences from other plants, two dominant fragments of approximately 360 bp were obtained from mango fruit. The unknown 3'-end of cDNAs were obtained by using 3'-RACE method. The PCR resulted in two fragments about 500 bp length with an overlap of 110 bp with the coding region of mango profilin. Full-length cDNA sequences were obtained by bridging the 3'-end and the corresponding gene fragments. The two cDNA sequences were submitted to the NCBI GenBank under the accession numbers DQ27054 (Man i 3.01) and DQ400579 (Man i 3.02).

The two entire cDNA length were both 660 bp containing a complete 396 bp open reading frame, a

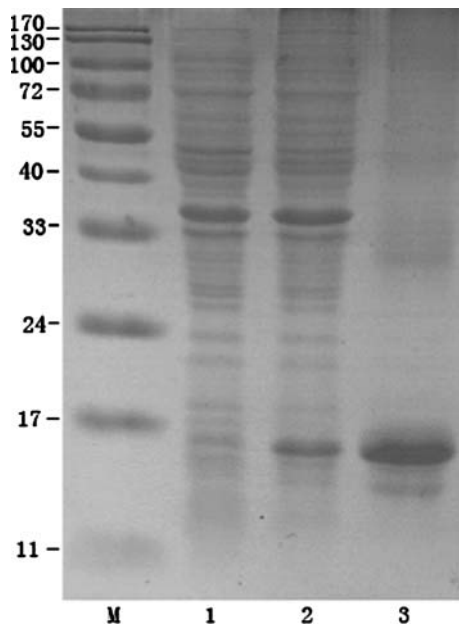


Fig. 3 Coomassie-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified recombinant mango profilin. M Molecular weight marker; lane 1 *E. coli*-lysate before induced; lane 2 after induced; lane 3 purified recombinant mango profilin

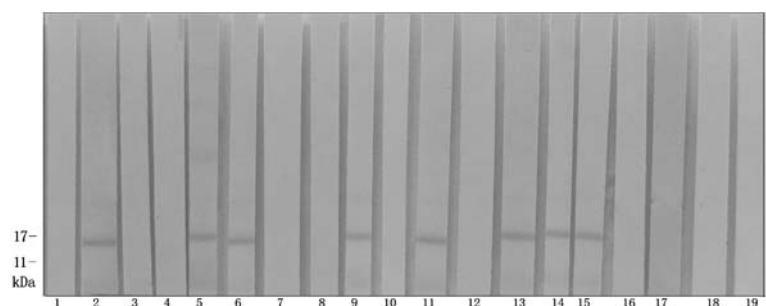
Sensitization to mango profilin

Serum samples taken from 18 patients which have adverse symptoms after ingestion of mango fruits and were suffering from pollinosis were investigated for IgE reactivity to recombinant mango profilin by immunoblot experiments. A pooled serum of 10 people with no allergic reaction was used as control. In the immunoblot experiments, IgE antibodies from 8 out of 18 serum samples (44%) reacted with the purified recombinant mango profilin (Fig. 4). No reaction was found with sera from control subjects. All sera with positive reactions to the mango fruit profilin have specific IgE to recombinant birch pollen profilin rBet v 2 in the ELISA.

Inhibition experiments

To investigate the IgE cross-reactivity to profilin from birch pollen, immunoblot inhibition and ELISA inhibition

Fig. 4 IgE-immunoblot of purified rMan i 3.02 with individual sera (1–18) from patients with allergy to mango and with the control serum (19)



experiments were carried out with the sera pool of mango allergic patients' sera that have positive reaction in the immunoblot experiments. In the immunoblot inhibition experiment, a complete inhibition of IgE binding to mango profilin was obtained by self-inhibition with mango recombinant profilin, moreover the recombinant birch pollen profilin rBet v 2 and mango extracts inhibited large part of the recombinant mango profilin. A similar inhibition pattern was obtained in the ELISA inhibition experiments (Fig. 5). A high degree of inhibition was also obtained with birch pollen profilin (about 78%) and mango extracts (85%). No inhibition occurred with the control inhibitor PBS buffer.

Discussion

Mango can cause severe anaphylactic reactions. Sensitization to mango fruit, as well as to mango seed and mango pollen, has been described. So far, only two major mango allergens have been previously identified [15]. However, cross-reactivities between mango fruit allergens and other fruits, vegetables, pollen, latex and nuts have been described [6–8, 16–18]. Paschke et al. [8] have studied the cross-reactivity between mango fruit, birch pollen extract and birch pollen major allergen Bet v 1, and found that the cross-reactivity was partly caused by the Bet v 1, the author assumed that there must have other allergens which were also partly responsible for the cross-reactivity between birch pollen and mango fruit because of the inhibition of IgE binding to mango fruit extract by birch pollen extract was 58% but the inhibition by Bet v 1 was only 28%. Henzgen et al. [18] suspected profilin of being responsible for the cross-reaction of mango fruits, but the role of profilin in the cross-reactivity of mango fruit was not finally clarified.

Profilin is a protein present in almost all eukaryotic cells. It was first identified as a minor allergen in birch pollen, so far, profilins were described as panallergens in botanically closely related [19] and distantly related plants or unrelated plant [20–23]. The aim of this study was to investigate the role of the panallergen profilin as a cross-reaction allergen in mango fruit. The cDNAs coding for

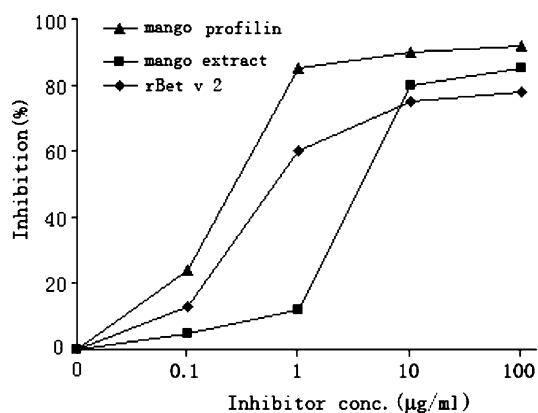


Fig. 5 ELISA inhibition experiments. Sera pool from mango allergic patients were preincubated with mango recombinant profilin, mango extract and rBet v 2

two different mango profilin isoforms have been isolated and sequenced. The two complete deduced amino acid sequences showed 88% of identity, both profilins consist of 131 amino acid residues with a calculated molecular mass of 14.97 and 13.99 kDa according to sequence data correlating with the molecular characteristics to the known plant profilins. Moreover, high degrees of amino acid sequence identities (73–90%) to previously described allergenic profilins from fruits, pollen and vegetables were found.

Man i 3.02 (Acc. Num.DQ400579), the isoform closer to profilins of others fruits such as pear (80%), peach (90%) and apple (80%), was induced into pet28(a) vector and expressed in *E.coli* and a high level of recombinant protein was obtained when protein synthesis was induced by IPTG at 37°C overnight. Highly purified Man i 3.02 was chromatographically isolated from the soluble fraction over a Ni²⁺ Sepharose affinity column.

The IgE reactivity of purified mango profilin was studied by immunoblot experiments. Eight sera out of 18 (44%) from patients with suspected allergy to mango fruits had IgE to mango profilin in the immunoblot experiments. Profilins have been identified as minor allergens in several plant species of tree, grass, and weed pollen and the profilin specific IgE was detected in 10–30% of pollen-allergic and food-allergic patients' sera [11], but recent studies showed that profilin was a major allergen in *Chenopodium album* pollen [24], melon [25] and date palm pollen [23]. Moreover, in date palm pollen allergy, profilin is not only a major allergen but an important allergen as well, as it is responsible for more than 70% of the IgE reactivity of the pollen extract [23]. About to the mango profilin studied here, the sensitization to mango profilin was found in 44% of mango fruits allergic patients' sera which is similar to the prevalence of IgE binding to banana profilin (44%) and pineapple profilin (42%) [26] and this may indicate that

profilin may represent important minor allergens in mango fruits.

The cross-reactivity of purified recombinant mango profilin (rMan i 3.02) with mango extract and rBet v 2 was determined by IgE-inhibition and ELISA inhibition experiments. The results revealed that the IgE reactivity of recombinant Man i 3.02 was largely inhibited by mango extract, this may indicate the similar IgE-binding capabilities of the natural profilin and the recombinant profilin. Immunoblot and ELISA inhibition experiments also revealed high cross-reactivity of the mango profilin with the profilin from birch pollen rBet v 2 (78%) and mango extracts (85%). The high inhibition levels in the cross-inhibition experiments may suggest that mango profilin shares epitope with profilin from different botanical origins.

Acknowledgments Authors thank the Shenzhen people's hospital for kindly sera supporting. This study was supported in part by National Natural Foundation (No. 30070702), Technological Key Program of Guangdong province (No. 2003A3080502) and Scholastic fund of Shenzhen University (2005028).

Reference

- Weinstein S, Bassiri-Tehrani S, Cohen (2004) Allergic contact dermatitis to mango flesh. *Int J Dermatol* 43:195–196
- Miell J, Papouchado M, Marshall AJ (1988) Anaphylactic reaction after eating a mango. *BMJ* 297:1639–1640
- Duque S, Fernández-Pellón L, Rodríguez F (1999) Mango allergy in a latex sensitized patient. *Allergy* 54:1004–1005
- Vargas Correa JB, Sanchez Solis L, Farfan Ale JA et al (1991) Allergological study of pollen of mango (*Mangifera indica*) and cross reactivity with pollen of piru (*Schinus molle*). *Rev Alerg* 38:134–138
- André F, André C, Colin L et al (1994) Role of new allergens and of allergen consumption in the increased incidence of food sensitizations in France. *Toxicology* 93:77–83
- Wellhausen A, Schöning B, Petersen A, Vieths S (1996) IgE binding to a new cross-reactive structure: a 35 kDa protein in birch pollen, exotic fruit and other plant foods. *Z Ernährungswiss* 35:348–355
- Brehler R, Theissen U, Mohr C et al (1997) 'Latex-fruit syndrome': frequency of cross-reacting IgE antibodies. *Allergy* 52:404–410
- Paschke A, Kinder H, Zunker K et al (2001) Characterization of cross-reacting allergens in mango fruit. *Allergy* 56:237–242
- Carlsson L, Nystrom LE, Sundkvist I et al (1977) Lindberg U. Actin polymerizability is influenced by profilin, a low molecular weight protein in non-muscle cells. *J Mol Biol* 115:465–483
- Valenta R, Duchene M, Pettenburger K et al (1991) Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* 253:557–560
- Radauer C, Hoffmann-Sommergruber K (2004) Profilins. In: Mills C, Shewry P (eds). Blackwell, Oxford
- Pastorello EA, Trambaioli C (2001) Isolation of food allergens. *J Chromatogr B* 756:71–84
- Bradford MM (1976) A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254

14. Stoscheck CM (1990) Quantitation of protein. *Methods Enzymol* 182:50–69
15. Paschke A, Kinder H, Zunker K et al (2001) Characterization of allergens in mango fruit and ripening dependence of the allergenic potency. *Food Agric Immunol* 13:51–61
16. Fernandez C, Fiandor A, Martinez-Garate A et al (1995) Allergy to pistachio: crossreactivity between pistachio nut and other Anacardiaceae. *Clin Exp Allergy* 25:1254–1259
17. Diaz-Perales A, Collada C, Blanco C et al (1999) Cross-reactions in the latex-fruit syndrome: a relevant role of chitinases but not of complex asparagines-like glycans. *J Allergy Clin Immunol* 104:681–687
18. Henzgen M, Rudeschko O, Schlenvoigt G et al (1998) Mangoallergie als Ausdruck einer Kreuzreaktion? *Allergologie* 7:338–343
19. Van Ree R, Fernandez-Rivas M, Cuevas M et al (1995) Pollen-related allergy to peach and apple: an important role for profilin. *J Allergy Clin Immunol* 95:726–734
20. Vallier P, Dechamp C, Valenta R et al (1992) Purification and characterization of an allergen from celery immunochemically related to an allergen present in several other plant species. Identification as a profilin. *Clin Exp Allergy* 22:774–782
21. Van Ree R, Voitenko V, Van Leeuwen WA et al (1992). Profilin is a cross-reactive allergen in pollen and vegetable foods. *Int Arch Allergy Immunol* 98:97–104
22. Scheurer S, Wangorsch A, Hausten D et al (2000) Cloning of the minor allergen Api g 4 profilin from celery (*Apium graveolens*) and its cross-reactivity with birch pollen profilin Bet v 2. *Clin Exp Allergy* 30:962–971
23. Asturias JA, Ibarrola I, Fernández J (2005) Pho d 2, a major allergen from date palm pollen, is a profilin: cloning, sequencing, and immunoglobulin E cross-reactivity with other profilins. *Clin Exp Allergy* 35:374–381
24. Bardenas R, Villalba M, Pascual CY et al (2004) Profilin (Che a 2) and polcalcin (Che a 3) are relevant allergens of *Chenopodium album* pollen: isolation, amino acid sequences, and immunologic properties. *J Allergy Clin Immunol* 113:1192–1198
25. López-Torrejón G, Crespo JF, Sánchez-Monge R et al (2005) Allergenic reactivity of the melon profilin Cuc m 2 and its identification as major allergen. *Clin Exp Allergy* 35:1065–1072
26. Reindl J, Rihs HP, Scheurer S et al (2002) IgE reactivity to profilin in pollen-sensitized subjects with adverse reactions to banana and pineapple. *Int Arch Allergy Immunol* 128:105–114