

Original article

Tomato profilin Lyc e 1: IgE cross-reactivity and allergenic potency

Background: To date, very little data are available about the nature of tomato allergens. Immunoglobulin E (IgE) cross-reactive profilins have been suggested to account for allergic symptoms in patients suffering from tomato allergy.

Methods: The cDNA of tomato profilin was amplified by reversely transcribed polymerase chain reaction (RT-PCR) from total RNA extracted from ripe tomato fruit. The gene was cloned into the pET101D expression plasmid and the protein was produced in *Escherichia coli* BL21. Purification was performed via poly-L-proline (PLP) affinity chromatography. IgE reactivity of recombinant tomato profilin was investigated by immunoblot and enzyme-linked immunosorbent assay. IgE-inhibition studies were performed to analyse cross-reactivity with other profilins. To determine the allergenic activity of the recombinant protein, basophil histamine release assays using sera of patients with adverse reactions to tomato were performed.

Results: Profilin was identified as a new minor allergen in tomato fruits. The recombinant tomato profilin comprises 131 amino acids and high sequence identity to other allergenic food and pollen profilins. It was shown to be IgE-reactive with a prevalence of 22% (11/50) in tomato-allergic patients. In patients with tomato allergy and multiple sensitization to other foods and birch pollen, IgE directed against tomato profilin showed a strong cross-reactivity with profilins from plant food sources and birch pollen. The tomato profilin was able to induce mediator release from human basophils.

Conclusion: The tomato profilin is a minor allergen in tomato fruit. Thus, it shows biological activity, as confirmed by *in vitro* histamine release assays with human basophils and thereby has the potential to account for clinical symptoms in tomato-allergic patients.

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To date, very little information is available about tomato (*Lycopersicon esculentum*) allergens. Most of the publications report on allergy to tomato linked to other allergies such as grass pollen (1) and latex allergy (2, 3). The prevalence of tomato allergy ranges from 1.5 to 16% among food-allergic populations, indicating that tomato is a relevant allergenic food. Recently, the glycosylated protein β -fructofuranosidase was identified as tomato allergen Lyc e 2 (4). The prevalence of an immunoglobulin E (IgE)-sensitization to Lyc e 2 is 17% among tomato-allergic patients. Interestingly, the IgE reactivity is completely determined by the glycan structure of Lyc e 2. A recombinant, non-glycosylated isoform expressed in *Escherichia coli* was not IgE-reactive at all. In a subgroup of patients, the glycan structure was also able to induce mediator release from human basophils confirming the biological activity of the sugar moieties (5). In addition to cross-reactive carbohydrate determinants, profilins are supposed to represent relevant IgE-binding

components in tomato. Profilins are ubiquitous proteins found in all eukaryotic cells where they function as actin and phosphatidylinositol 4,5-bisphosphate binding proteins involved in the regulation of the actin cytoskeleton and signal transduction (6). They are highly conserved and were identified as allergens of many grasses, weeds, trees, fruits and vegetables (7) as well as of natural rubber latex (8). The presence of profilin-specific IgE antibodies is a reason for cross-reactivity to botanical unrelated allergen sources, and therefore, profilins are termed panallergens. Foetisch et al. (9) have shown that profilin of tomato probably plays a role in IgE reactivity to this food source, using recombinant profilin from birch Bet v 2 (10) as inhibitor of IgE binding to tomato extract.

In the present study, the cDNA of tomato profilin was cloned and expressed in *E. coli*. The recombinant non-fusion protein was purified via PLP affinity chromatography and tested for its IgE reactivity in immunoblot and enzyme-linked immunosorbent assay (ELISA)

studies. Moreover, the cross-reactivity to other food and pollen profilins was investigated using ELISA inhibition. The ability to induce type I reaction was shown by mediator release using passively sensitized human basophils. The profilin was designated as allergen Lye 1 according to the IUIS allergen nomenclature sub-committee (see <http://www.allergen.org>).

Materials and methods

Preparation of allergen extract

Extract from ripe tomato fruit was prepared by a low-temperature method as previously described (11). In brief, pieces of fresh fruit were frozen in liquid nitrogen, and ground in a mill without thawing. The obtained powder was homogenized in pre-chilled acetone and stored overnight at -20°C . The precipitate was filtered, washed twice with ice-cold acetone and once with acetone : diethylether (1 : 1, v/v) and lyophilized. Extraction of proteins from this powder was done with phosphate buffered saline (PBS), (0.15 M NaCl/0.01 M NaH_2PO_4) at 4°C . After centrifugation, the supernatant was collected, filtered and freeze-dried. The lyophilized extract was stored at -80°C .

Amplification of *L. esculentum* profilin cDNA

Total RNA was isolated from tomato fruit using the 'RNeasy Plant RNA Mini Kit' (Qiagen, Hilden, Germany). DNA contaminations were removed by using the 'RNase-free DNase set' (Qiagen, Hilden, Germany). The RNA was reversely transcribed (RT) with the 'first strand cDNA synthesis kit' (Amersham Pharmacia Biotech, Freiburg, Germany) according to the manufacturer's instructions using 1 μg total RNA for each transcription and the NotI-d(T)₁₈ oligonucleotide for priming. To obtain the complete coding region, the RT products were amplified using gene specific 5' and 3' primers selected on the basis of the published sequences for tomato pollen profilin. Primers LeProFW1, matching with the N-terminal sequence of the coding region: 5'ATGTCGTGGCAAACATATGTCGATG, and LeProRev1, matching with the C-terminal sequence of the coding region: 5'TTAATATCCCTGATCAACAAGGTAATC. For amplification the 'expand long template DNA polymerase set' (Roche, Mannheim, Germany) was used. The polymerase chain reaction (PCR) conditions were the following: 94°C , 5 min, followed by 30 cycles: 94°C , 30 s; 50°C , 30 s; 68°C , 2 min. The final extension was 7 min at 68°C . The cDNA obtained was cloned into the pCRII-TOPO vector (Invitrogen, Groningen, The Netherlands) and transformed in *E. coli* TOP10. Obtained clones were analysed by sequencing.

DNA sequencing

The sequence analysis was carried out with an ABI 373 automated fluorescent sequencer (Applied Biosystems, Foster City, CA) using vector or gene specific primers and the 'ABI PRISM BigDye Terminators v3.0 CycleSequencing Kit' according to the manufacturer's instructions.

Expression and purification of recombinant Lye 1

For protein expression in *E. coli*, the coding region was cloned into the pET101D vector using the 'pET directional TOPO expression kit' (Invitrogen, Groningen, The Netherlands). The DNA was

amplified using a 5' primer containing the sequence CACC for directional cloning, LeProFWCACC: 5'ATGTCGTGGCAAACA TATGTCGATG and a 3' primer containing a stop codon to avoid expression of the C-terminal histidine tag of the vector LeProRev2: 5'CTAGAGACCCTGTTCAATGATATAGT. The PCR was carried out using Vent DNA polymerase (New England Biolabs, Frankfurt a.M., Germany) under the following conditions: 94°C , 5 min, followed by 30 cycles: 94°C , 30 s; 50°C , 30 s; 72°C , 2 min. The final extension was 7 min at 72°C .

The pET101D constructs were transformed in *E. coli* BL21 star cells (Invitrogen, Groningen, The Netherlands) and protein synthesis was induced with 1 mM isopropyl β -D-thiogalactopyranoside for 5 h at 37°C . After induction, bacteria were harvested by centrifugation and stored at -80°C . The expressed protein was purified via PLP-Sepharose chromatography as described elsewhere (12, 13).

Patients' sera

Serum samples were taken from a group of 50 patients with a positive case history of immediate type reactions to tomato fruit. Out of these, eight patients were recruited from Spain, the others were from Germany. Only adults were included in the study, the age ranged between 19 and 65 years and 20% were male. Most of the patients ($n = 33$) reported mild oral allergy syndrome (OAS), whereas systemic reactions were less frequent ($n = 17$). Twenty-two of the patients underwent skin prick testing (SPT) with commercial tomato extract, 14 (64%) showed positive skin reaction. Four patients with positive SPT were not reactive with tomato allergens in CAP-FEIA (Pharmacia Diagnostics, Uppsala, Sweden) tests (Table 1). Serum from a German non-allergic subject was taken as a negative control.

Determination of specific IgE

Measurement of tomato-specific IgE was performed with the CAP FEIA system according to the manufacturer's instructions.

IgE-immunoblotting and immunoblot inhibition

Allergen extracts (20 $\mu\text{g}/\text{cm}$) and purified recombinant profilins (0.5 $\mu\text{g}/\text{cm}$) were separated by sodium dodecylsulfate polyacryla-

Table 1. Clinical data of 50 patients with positive case history after ingestion of tomato investigated in this study

CAP	Symptoms			
	Mild (OAS)		Systemic (urticaria, ND, nausea, anaphylaxis)	
	CAP (<i>n</i>)	SPT pos.	CAP	SPT pos.
0	5	1/3	11	3/3
1	3	0/0	2	0/1
2	16	5/8	1	0/0
3	7	3/4	3	1/2
4	2	1/1	0	0/0
5	0	0/0	0	0/0
Total	33	10/16	17	4/6

Abbreviations: OAS, oral allergy syndrome; ND, neurodermatitis; *n*, number of patients investigated; SPT pos, patients with positive skin prick test/patients tested.

mid gel electrophoresis (SDS-PAGE) under reducing conditions as described by Laemmli et al. (14) in a Mini-Protein 3 cell (BIO-RAD, Munich, Germany). For immunoblot analysis, proteins were transferred onto 0.45 µm nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) by tank blotting using the BIO-RAD Mini Trans blot cell for 1 h at 300 mA and membrane was blocked in TBS/0.3% Tween-20 and cut into 3 mm wide strips. After incubation with 1 : 10 diluted patients' sera, immunostaining of bound IgE antibodies was performed with an alkaline phosphatase conjugated anti-human-IgE antibody (Pharmingen, Hamburg, Germany, 1 : 750 dilution, 4 h) and the 'BIO-RAD alkaline phosphatase conjugate substrate kit' (BIO-RAD).

For inhibition of IgE-binding to recombinant tomato profilin, a pooled patient serum (1 : 10 diluted) was preincubated with tomato extract (0.0125, 0.125, 1.25, 12.5 and 125 µg/ml) and the recombinant tomato profilin (0.001, 0.01, 0.1, 1.0 and 10 µg/ml), respectively.

ELISA, EAST and IgE inhibition

For ELISA, 96 well Maxisorb plates (Nunc GmbH & Co. KG, Wiesbaden, Germany) were coated overnight at 4°C with 0.5 µg purified protein in PBS, 100 µl per well. Plates were blocked in PBS/1% bovine serum albumin (BSA) for 1 h at room temperature (RT). Plates were washed three times with PBS/0.05% Tween-20 before incubation with the patients' sera and diluted 1 : 2 in PBS/0.05% Tween-20/0.3% BSA, 100 µl per well. Incubation was performed overnight at RT. Plates were washed again, incubated with the first antibody, rabbit anti-human-IgE, 1 : 4000 (DAKO Diagnostika, Hamburg, Germany), for 1 h. Next incubation was performed with the second antibody, goat-anti-rabbit-biotin, 1 : 6000 (DAKO Diagnostika), 1 h at RT. In the last step, streptavidin was coupled to horse radish peroxidase (Calbiochem-Novabiochem, Bad Soden, Germany), 1 : 10 000 that was left for 30 min at RT in the dark. Each incubation step was followed by a three-times washing step with PBS/0.05% Tween-20. After visualization of the enzymatic activity with tetramethylbenzidine (TMB) as substrate at 37°C for 20 min, the reaction was stopped by addition of 50 µl 3 M H₂SO₄ and absorption was measured at 450 nm (15). Duplicate determinations were performed in each case.

For ELISA inhibition, sera were preincubated for 30 min at RT with 0.1, 1 and 10 µg/ml of the specific inhibitor protein before overnight incubation on the allergen-coated plates. The major allergen of celery, Api g 1 (Acc. No. Z4896) was used as non-specific control allergen in the highest concentration (10 µg/ml). Inhibition rates were calculated as percentage of the non-inhibitor values of the sera under consideration of the non-specific binding control (no antibodies and serum).

Allergen-specific IgE was quantified by the EAST according to the manufacturer's instructions (Allergopharma Spez. IgE ELISA, Allergopharma, Reinbek, Germany). Briefly, tomato extract was coupled to cyanogen bromide activated filter paper disks (10 µg protein per disk, Hycor, Kassel, Germany). Dose-related EAST inhibition experiments were performed by preincubation of patient sera (1 : 2 dilutions) with 50 µl rLyc e 1 (10 and 100 µg/ml) and 50 µl tomato extract (500 µg/ml). Inhibition values were calculated by measurement of optical densities (ODs) as follows:

$$\% \text{ inhibition} = [\text{OD}(\text{B}_0) - \text{OD}(\text{P})] / [\text{OD}(\text{B}_0) - \text{OD}(\text{NSB})] \times 100$$

where OD(B₀), OD(P), and OD(NSB) represent values for serum, serum with inhibitor and nonspecific binding, respectively.

Basophil histamine release

The histamine release was performed as described (16) with several modifications. Peripheral blood was drawn from non-allergic donors and peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Histopaque® (Sigma, Taufkirchen, Germany) centrifugation. The conditions for stripping of the nonspecific IgE and for the passive sensitization procedure were chosen according to the recommendations of Pruzansky et al. (17). Cells sensitized with a non-allergic serum served as negative control. Stimulation of the cells was performed using a histamine kit (Immunotech, Marseille, France) according to the manufacturer's instructions with ten-fold dilutions of the allergens starting at 10 µg/ml. The histamine releases were measured by an enzyme immunoassay. After subtraction of the spontaneous release of the basophils, the allergen-induced histamine release was calculated as percent of the total amount of histamine determined after lysis of the basophils by twofold freezing and thawing of the cells. A histamine release of more than 10% was considered positive. Duplicate determinations were performed in all cases.

Results

Cloning of the cDNA, recombinant expression and molecular characterization of profilin from tomato fruit

After sequencing of the cDNA obtained with the primers based on the tomato pollen profilin sequence LePro1 (Acc. No. U50195), we found that the 3' primer matched in the 3' UTR of the fruit profilin sequence (Acc. No. AY061819). We speculate that the profilin cDNA was amplified by the application of a highly specific forward primer (LePrFW1) matching in the conserved N-terminal consensus sequence of the profilin family (MSWQT) and an unspecific reverse primer matching randomly to a homologous sequence in the 3'-UTR. Interestingly, the amino acid sequences from both profilins show an identity of only 78%, and the pollen profilin contains two additional amino acids (Fig. 1). For expression of the recombinant tomato fruit profilin, the complete coding cDNA sequence including the start and stop codons was cloned into the pET101D expression vector. In general, this vector provides a C-terminal histidine tag, but inserting the stop codon at the C-terminus prevents fusion of this tag to the protein. The non-fusion protein was purified by affinity chromatography using a PLP-Sepharose column to high purity (Fig. 2A). Since the IgE-binding to recombinant tomato profilin was completely inhibited by preincubation of a pooled serum from five patients (numbers 10, 13, 28, 39 and 47) with sensitization to tomato profilin (Fig. 3) by tomato extract (12.5 µg/ml), it was demonstrated that natural tomato profilin comprises all IgE-epitopes of the recombinant molecule (Fig. 2B).

Sensitization to tomato profilin

Serum samples taken from patients with adverse reactions to tomato were investigated for IgE binding to recombinant tomato profilin in immunoblot as well as ELISA

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rLyc e 1      MSWQTYVDDHLMCDIEGN--HLTSAAIIGQDGSVWAQSANFPQFKPEEITAIMNDFAEPG 58
LePro1       MSWQTYVDDHLMCDIEGTGHHLSSAAILGFDGSVWAQSPNFPKFKAAEITNIMKDFDEPG 60
              *****.* **.:****:* *****.****:*.**** **:* **
rLyc e 1      TLAPTGLHLGGTKYMWIQGEAGAVIRGKKGAGGITVKKTNQALIGIYDEPMTPGQCNCMI 118
LePro1       HLAPTGLFLAGTKYMWIQGEPGAVIRGKKGPGGITIKKTAQALIFGVYEEFVTPGQCNCMV 120
              *****.*.*****.*.*****.*.***:*** *****:*.*:*.*****:
rLyc e 1      VERLGDYIIEQGL 131
LePro1       VEKIGDYLVDQGY 133
              **..*****.*

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Figure 1. ClustalW sequence alignment (<http://www.ebi.ac.uk/clustalw/>) of amino acid sequences of recombinant tomato fruit profilin rLyc e 1 and of the tomato pollen profilin Le Pro 1. Numeric order starts with the first amino acid methionine. Identical amino acids are indicated by “*”, “:” indicates conserved substitutions, “.” indicates semiconserved substitutions. Underlined sequence shows two additional amino acid residues presented in the pollen profilin sequence.

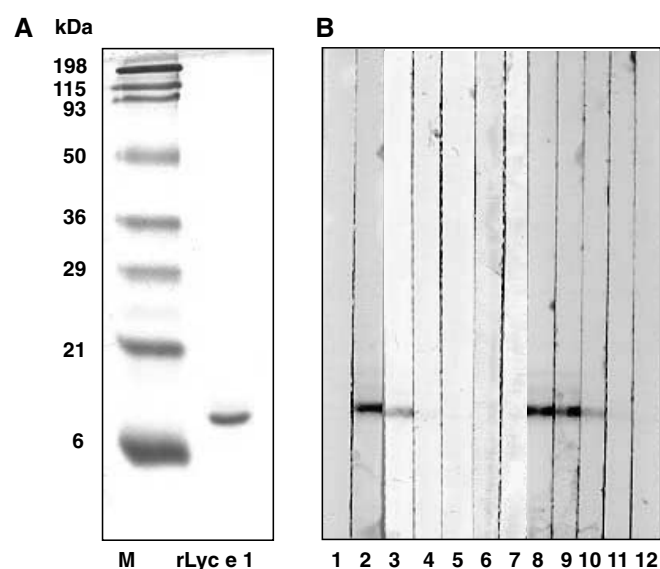


Figure 2. (A) Purification of recombinant *Lyc e 1*. SDS-PAGE analysis of recombinant *Lyc e 1* after affinity purification with poly-L-proline and dialysis. Coomassie stain. M: Molecular weight marker. (B) Inhibition of IgE-binding to r*Lyc e 1* after preincubation of a pooled serum from tomato allergic patients with r*Lyc e 1*, 0.001 $\mu\text{g/ml}$ (3), 0.01 $\mu\text{g/ml}$ (4), 0.1 $\mu\text{g/ml}$ (5), 1.0 $\mu\text{g/ml}$ (6) and 10 $\mu\text{g/ml}$ (7), and tomato extract, 0.0125 $\mu\text{g/ml}$ (8), 0.125 $\mu\text{g/ml}$ (9), 1.25 $\mu\text{g/ml}$ (10), 12.5 $\mu\text{g/ml}$ (11), 125 $\mu\text{g/ml}$ (12), buffer control (1), without inhibitor (2).

experiments. In the immunoblots, 11 of 50 patients (22%) reacted with the purified recombinant tomato profilin (Fig. 3). The prevalence of IgE-binding was confirmed by ELISA. Profilin sensitization was diagnosed in 13 patients (26%) (not shown). All patients with IgE reactivity to blotted profilin were detected positive in the ELISA. Therefore, the protein was designated Lyc e 1 according to the regulations of the IUIS allergen nomenclature sub-committee (<http://www.allergen.org>).

IgE binding to tomato extract coupled onto paper discs was inhibited by preincubation of patient sera with

purified Lyc e 1 and tomato extract. IgE reactivity to tomato extract was inhibited by 24% (patient 28), 25% (patient 39) and 42% (patient 47) after preincubation of the respective sera with rLyc e 1 (10 µg/ml). In contrast, IgE-binding to tomato allergens was nearly completely abolished with the tomato extract used as positive control (patient 28: 96.6%, patient 39: 100%, patient 47: 86.1%) (not shown). Therefore, IgE reactivity to tomato profilin comprises 24% up to 42% of the overall IgE reactivity to tomato extract in preselected patients with strong IgE reactivity to purified rLyc e 1 (Fig. 3), all with CAP-classes from 2 to 3 to tomato.

Cross-reactivity of profilins from different allergenic sources

Sera of rLyc e 1-positive tomato allergic patients with birch pollinosis and other food allergies, were analysed in respect of cross-sensitization with allergenic profilins from birch (Bet v 2) (10), celery (Api g 4) (18), sweet cherry (Pru av 4) (13), pineapple (Ana c 1) (19), banana (Mus xp 1) (19) and carrot (Dau c 4) (Acc. No. AF456482). IgE-immunoblotting experiments revealed that all patients were sensitized to rBet v 2 and the respective recombinant food profilins, except for patient 10 reporting an allergy to banana without sensitization to rMus xp 1 (not shown). Sera from patients with known sensitization against the specific profilin were used as positive controls (not shown).

To further investigate the IgE cross-reactivity to profilins from different food sources, dose-dependent ELISA inhibition experiments were performed with rBet v 2, rApi g 4, rPru av 4, rAna c 1 and rDau c 4. Sera from two patients with birch pollinosis, and additional food allergies to celery and banana (patient no. 10) and to celery, pineapple, cherry and carrot (patient no. 28) were selected for inhibition of IgE binding to rLyc e 1 coupled to ELISA plates. Only those profilins that we suspected to cause clinical symptoms were used as inhibitors. Complete inhibition was obtained with 10 µg/ml rLyc e 1 used as positive control. For serum 10, almost complete inhibition (93%) of IgE binding to

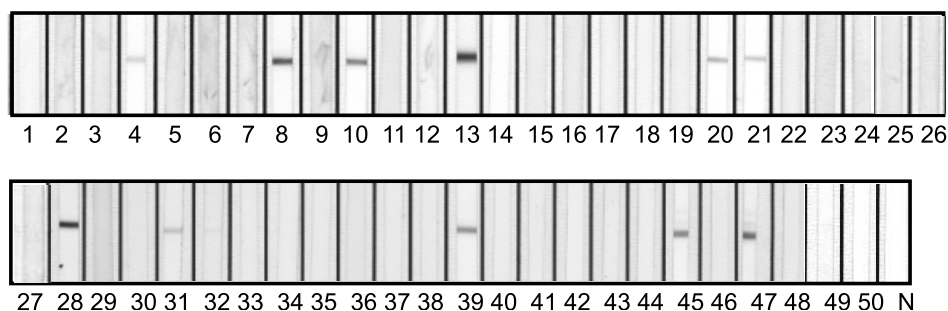


Figure 3. Immunoblot showing IgE reactivity of tomato allergic patients to purified rLyc e 1. N: negative control, serum from non-allergic subject. Sera no. 10 and 28 were selected for analysis of cross-reactivity and sera no. 10 and 13 were applied to determine the allergenic potency of tomato profilin.

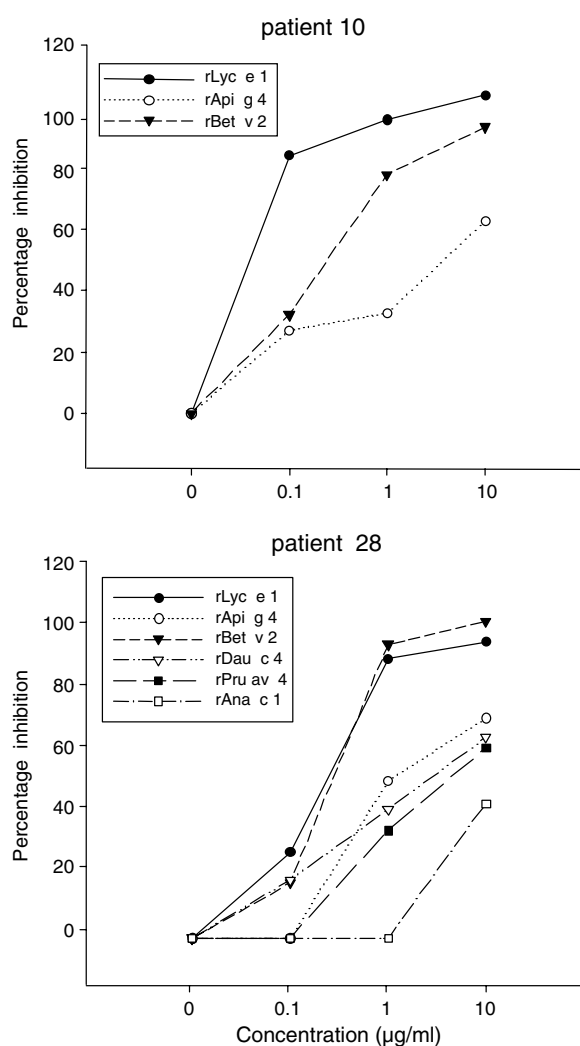


Figure 4. ELISA inhibition experiments with sera no. 10 and no. 28. Experiments were performed in a dose-dependent manner with rLyc e 1 on the solid phase. Sera were preincubated with rLyc e 1, rBet v 2, rApi g 4, rPru av 4, rDau c 4 and rAna c 1 as indicated. Inhibition with an irrelevant allergen (Api g 1) was used as negative control (not shown).

rLyc e 1 was obtained with the highest concentration of rBet v 2, whereas 10 µg/ml rApi g 4 revealed inhibition of only 63%. For serum 28, preincubation with rBet v 2 resulted in a complete inhibition of IgE binding in the highest concentration. Inhibition with rDau c 4, rApi g 4 and rPru av 4 was almost in the same range (60–70%) at 10 µg/ml, whereas the highest concentration of rAna c 1 revealed only 44% inhibition of IgE binding. All observed inhibition values were dose-dependent (Fig. 4). No or very weak inhibition was observed with the non-related allergen from celery, Api g 1, in the highest concentration (not shown).

Basophil histamine release

In order to confirm the allergenic activity of tomato profilin, its ability to induce histamine release from human basophils was investigated. We performed histamine release experiments with stripped basophils from nonallergic donors, passively sensitized with sera from tomato-allergic patients no. 10 and 13. Tomato extract and recombinant tomato profilin rLyc e 1 were used in these assays and BSA was used as negative control. Both sera reacting with the tomato profilin revealed histamine release in a range of 18–40% in a dose-dependent manner (Fig. 5). Histamine release of up to 30% (patient 10) or 55% (patient 13) was obtained with the highest concentration of tomato extract, but there was no release obtained with the negative control (not shown).

Discussion

The tomato allergen Lyc e 1 belongs to the conserved family of profilins. In this study, we cloned the coding sequence of this protein from cDNA obtained from ripe tomato fruit and expressed it in *E. coli*. The recombinant protein comprises 131 amino acid residues with a calculated molecular weight of 14.08 kDa and a pI of 4.71. It shows a very high sequence identity of 76–86% to other allergenic plant profilins such as Pru av 4 from

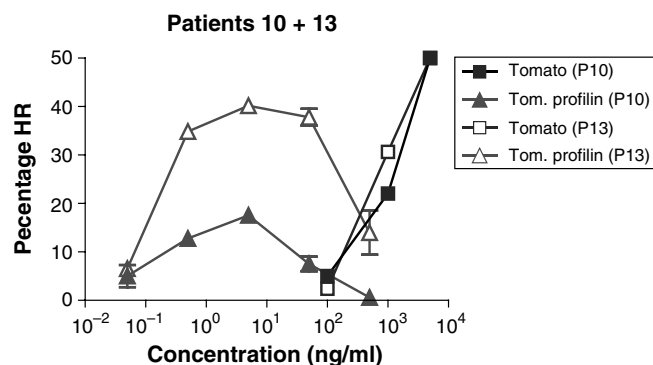


Figure 5. Induction of histamine release from stripped human basophils passively sensitized with sera from tomato-allergic patients, no. 10 (P10) and 13 (P13). Cross-linking was performed with rLyc e 1 and tomato extract and BSA was used as negative control (not shown).

cherry (13), Ana c 1 (19) from pineapple, Mus xp 1 from banana (19), Dau c 4 from carrot (Acc. No. AF456482), Api g 4 from celery (18) and Bet v 2 from birch (10). The first report on recombinant tomato profilin was from Yu et al. (20) who cloned the profilin from tomato pollen. The amino acid sequence identity between tomato pollen and fruit profilin is only 78% and the pollen sequence comprises two additional amino acids. A blast search in GenBank revealed another sequence from tomato fruit profilin Lyc e 1 (Acc. no. AJ417553), but both sequences show only 89% identity, indicating the existence of different profilin isoforms in tomato fruit. At the same time, the sequence of bell pepper profilin Cap a 1 (Acc. no. AJ417552) was published by the same group. Interestingly, the sequence identity of 94% between the identified profilin and that from bell pepper is higher than that of both tomato sequences. This may be explained by the fact that both, tomato and bell pepper, are in a tight taxonomic vicinity.

The IgE reactivity of rLyc e 1 and its prevalence among tomato-allergic patients was investigated by means of immunoblotting and ELISA. The ELISA was a little more sensitive, 26% of the investigated patients reacted with rLyc e 1 in this assay in contrast to 22% who showed positive reaction in the immunoblots. Probably some epitopes are masked in the immunoblot due to denaturing conditions. The prevalence of less than 50% indicates that tomato profilin should be classified as a minor allergen of tomato fruit. The frequency of IgE reactivity obtained in our study is in good agreement with those reported for profilins from other plants species such as birch-pollen (21) and carrot (22) with frequencies of about 20%.

Using a cross-reactive polyclonal rabbit antiserum directed against profilin Pyr c 4 from pear (23) for immunoblotting, we could confirm that natural profilin can be detected in tomato extract (not shown). Moreover, the existence of profilin as an IgE-reactive component of

tomato extract was demonstrated by inhibition of IgE-binding to rLyc e 1 after preincubation of patient sera with tomato extract. We, therefore, confirm the presence of a profilin isoform with all IgE-epitopes of the recombinant molecule within the natural extract. The proportion of tomato allergen-specific IgE, which binds tomato profilin, can comprise 24 to up to 42% in preselected patients with strong IgE reactivity to Lyc e 1. These patients, as the majority of tomato-allergic patients, are characterized by sensitization to several tomato proteins, that is, high molecular weight glycoproteins with cross-reactive carbohydrate determinants and a protein with a molecular weight of approximately 10 kDa (patient 28) or Lyc e 2 (patient 39) (data not shown).

Profilins are described as panallergens in a wide variety of plant sources (8, 13, 19, 24–28). To determine the cross-reactivity of rLyc e 1 with other allergenic food profilins and with pollen profilin Bet v 2, we investigated sera from Lyc e 1-sensitized tomato-allergic patients with birch pollinosis who complained about other food allergies, with regard to their IgE reactivity to profilins. In a western blot experiment, we could show that serum no. 10 reacts with Api g 4 from celery, and Bet v 2 from birch. Unexpectedly, no binding to banana profilin was observed even though the patient had adverse reactions to banana. Interestingly, rLyc e 1 and rMus xp 1 revealed 80 and 78%, respectively, amino acid sequence identity to rBet v 2. So, in this case, banana and tomato profilin seem to have different IgE epitopes, even though their amino acid sequences show very high identity. To further analyse the IgE cross-reactivity, we performed ELISA inhibition studies with sera no. 10 and 28 which clearly showed that the profilins exhibited different inhibition potencies. In both cases, besides Lyc e 1 the birch pollen profilin Bet v 2 had the highest inhibition potency, whereas the other investigated food profilins inhibited the IgE reactivity by 45 to up to 70% at the highest concentration. It was already shown that profilins from different plant sources, for example, Api g 4 and Bet v 2, reveal differences in their IgE epitopes (13, 18). Our results are in accordance with these observations.

Our histamine release experiments with rLyc e 1 demonstrated that the tomato profilin Lyc e 1 is a relevant inducer of mediator release from human basophils, thus confirming biological activity of this protein and indicating that profilin can be a clinically relevant allergen accounting for allergic symptoms in tomato-allergic patients.

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