

The major allergen of peach (*Prunus persica*) is a lipid transfer protein

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Background: Allergy to fresh fruits and vegetables is mostly observed in subjects with pollinosis, especially from birch, because of cross-reacting allergens in vegetable foods and pollens. However, allergic reactions to fruits, specifically Rosaceae fruits, have been reported in subjects without pollinosis. **Objective:** This study evaluated the pattern of IgE reactivity, identifying the allergen responsible in 2 groups of patients with oral allergy syndrome to peach with or without birch pollinosis.

Methods: The allergenic components of peach were detected by SDS-PAGE and immunoblotting. The major peach allergen was purified by HPLC with a cation-exchange column followed by gel filtration chromatography. Its IgE-binding capacity and its homology with the protein of the crude extract were demonstrated by immunoblotting inhibition techniques. To better characterize this allergen, periodic acid-Schiff stain and isoelectrofocusing were used. The amino acid sequencing was done with a gas-phase sequencer.

Results: SDS-PAGE and immunoblotting of the 15 patients allergic to peach, 8 without and 7 with birch pollinosis, showed that they all recognized a protein with a molecular weight of 9 kd. This was the only allergen recognized by patients not sensitized to pollen, whereas the birch pollen-sensitive patients had IgE binding to other allergenic proteins at higher molecular weights. The purified 9-kd protein retained its IgE-binding capacity, was negative to periodic acid-Schiff stain, and had an isoelectric point value of greater than 9. A search in the Swiss Prot Bank showed this was a lipid transfer protein, belonging to a group of molecules involved in the defensive system of plants.

Conclusions: The major allergen of peach is a 9-kd protein belonging to the group of lipid transfer proteins. This is the only allergen recognized by patients allergic to peach but not sensitized to birch pollen. (*J Allergy Clin Immunol* 1999;103:520-6.)

Key words: Oral allergy syndrome, peach, birch pollinosis, immunoblotting, major allergen, lipid transfer protein

Abbreviations used

LTP: Lipid transfer proteins
MW: Molecular weight
OAS: Oral allergy syndrome
PAS: Periodic acid-Schiff
pI: Isoelectric point
SPT: Skin prick test

The mechanisms underlying the development of allergy to fruits and vegetables are not yet fully clear. Cross-sensitization by the respiratory route to homologous structures in pollens and in vegetable foods is likely to be involved, giving rise to the well-known associations between pollinosis from birch, grass, and mugwort and allergic reactions to given vegetable foods.¹ For the association between birch hay fever and oral allergy syndrome (OAS) to foods such as apple, hazelnut, celery, carrot, and others, the immunochemical basis is the sharing of components highly homologous to Bet v 1, the major birch allergen, which belongs to the so-called family of pathogenesis-related proteins.² However, in recent years sensitization to fruits has appeared that has not been significantly associated with birch hypersensitivity, although arising in patients with other kinds of pollinosis and in some cases in atopic patients not sensitized to pollens.³ This suggests that unidentified vegetable-specific allergens may be involved in these sensitizations and that the route of sensitization may not be inhalation. In our experience Prunoideae fruits, including peach, are the ones most involved in allergic reactions that do not seem related to any kind of pollinosis.³ Peach is one of the most frequent causes of food allergy in Europe, being reported as a culprit food both in the Northern regions⁴ and in the Mediterranean area, where it is especially associated with OAS.⁵ A recent study from Israel⁶ used double-blind challenges to verify the role of single food items in allergic reactions in an adult population (including nasal, pulmonary, and cutaneous symptoms), and peach accounted for the highest number of reactions.

We previously identified the major allergen of peach as a low molecular weight (MW) protein shared by fruits belonging to the botanical subfamily of Prunoideae (eg, apricot, plum, and cherry) but not by birch and grass pollens.⁷ The present study was aimed at characterizing this allergen by purification, determination of isoelectric point (pI) value, amino acid sequencing, assessment of glycosylation, and determination of biologic function.

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The IgE-binding activity of the purified fractions was checked by using sera from patients with peach-specific IgE antibodies and clinical allergic reactions to peach.

METHODS

Patients

Patients referred to the Allergy Center of the 3rd Department of General Medicine of the University of Milan and to the Bizzozzero Division of Niguarda Ca' Granda Hospital in Milan were selected on the basis of documented allergic reactions to peach. All those who complained of symptoms when eating peach were recruited and studied by a protocol including the following: (1) a careful clinical history of the kind of allergic reactions, with a review of medical records if severe, life-threatening reactions had occurred; (2) skin prick tests (SPTs) with fresh fruit performed by means of the prick-prick method⁸; (3) measurement of peach-specific IgE antibodies by means of the CAP System (Pharmacia-Upjohn, Uppsala, Sweden); and (4) an open food challenge with fresh peach performed by having the patient chew increasing amounts of the fruit, ranging from 4 to 64 g, as previously reported.⁷

Allergy to pollens was assessed from a clinical history of respiratory symptoms during the pollen season and positive SPT and CAP system results with commercial extracts of pollens from birch and grasses.

Blood was drawn from all patients, and sera were collected and stored at -80°C . The sera were used to identify the major and minor allergens of peach.

Identification of peach allergens

Peach extract. To prepare peach extract we followed the method of Björkstén et al.⁹ We used fresh peach peel, which on the basis of our previous observation (unpublished data) has a higher protein content than the pulp. A given amount of peach peels were diluted 5:1 (wt/vol) in 10 mmol/L PBS (pH 7) with 2% solid polyvinylpyrrolidone, 2 mmol/L EDTA disodium salt, 10 mmol/L sodium diethyldithiocarbamate, and 3 mmol/L sodium azide (NaN_3). After homogenizing and centrifuging at 16,000 rpm at 4°C for 30 minutes, the supernatant was dialyzed against 10 mmol/L PBS (pH 7) with 3 mmol/L NaN_3 for 48 hours at 4°C , changing the buffer at 16- to 18-hour intervals. Visking dialysis tubing (Serva Boehringer Ingelheim, Heidelberg, Germany) with a MW cutoff of 8000 d were used. The extract was centrifuged at 3000 rpm for 5 minutes, stocked, and frozen at -20°C . For each experiment, we handled about 300 g of peels in 60 mL of PBS. We thus obtained about 100 mL of final extract each time, with a protein content ranging from 6 to 9 mg/mL, as assessed by the colorimetric method of Lowry et al¹⁰ using Folin reagent, which is suitable for high-protein concentration extracts and reading with a spectrophotometer at 700 nm. This extract was used for SDS-PAGE experiments, but it was too diluted for allergen purification and was therefore further concentrated by centrifugation at 3000 rpm at 4°C for 7 hours by using a Centriprep 3 concentrator (Amicon, Inc, Beverly, Mass) with a MW cut-off of 3000 d. The extract was concentrated 10 times to achieve a final concentration of about 60 to 70 mg/mL, as calculated by the method reported above.

SDS-PAGE. SDS-PAGE of total peach extract was run in a discontinuous buffer system by using the method of Neville,¹¹ with a separation gradient gel of 7.5% to 20% and a 6% stacking gel. The sample at the protein concentration of 6 to 9 mg/mL was diluted 1:2 in sample buffer containing Tris- SO_4 (pH 6.1), SDS (10%), 2-mercaptoethanol, glycerol (50% vol/vol), and Bromphenol blue (1%). The samples were denatured at 100°C for 5 minutes and centrifuged at 10,000 rpm for 5 minutes. In the same gel reference markers were run (Pharmacia-Upjohn) at known MWs (ie, 94, 67, 43, 30, 20.1, and 14.4 kd). Peach extracts (0.280 mg/cm gel) were run at 6 mA

for 16 hours in a BIO-RAD Protein IIxi vertical electrophoresis slab cell (BIO-RAD Laboratories).

After separation, a part of the gel was fixed and stained with Coomassie Brilliant Blue R-250 (Pharmacia-Upjohn), and another part was used for immunoblotting studies.

Detection of IgE-binding capacity by immunoblotting. Immunoblotting was done by electrophoretically transferring the separated proteins to a nitrocellulose membrane (Amersham, 0.45 μm) by using a trans-blot cell from BIO-RAD at 4°C , 0.45 Å, and 100 V for 4 hours. The unoccupied protein-binding sites in the nitrocellulose membrane were blocked by 30 minutes of incubation at 37°C with PBS (pH 7.4 ± 0.2) and 0.1% Tween 20. The nitrocellulose was then cut into strips, which were incubated overnight at room temperature with the serum of each patient. Binding by specific antibodies was detected by incubation with ^{125}I -labeled anti-human IgE diluted 1:4 in blocking solution for 6 hours at room temperature and exposure on x-ray film at -70°C for 2 days.

As we have previously demonstrated,⁷ the 9-kd protein was the major peach allergen, and therefore we tried to purify and identify this protein. To assess the IgE-binding capacity of the 9-kd purified protein, immunoblotting was done with pooled sera from the patients allergic to peach; the nonspecific IgE binding was evaluated by immunoblots with sera from 6 atopic patients not allergic to peach. The first 3 patients had low total IgE levels, and the other 3 had high total IgE levels. The serum of 1 patient allergic to peach was used as positive control. One immunoblot was done without addition of serum (ie, with buffer control), and another was done with serum from 1 nonallergic individual.

Purification of the peach 9-kd allergen by HPLC

Cationic exchange. The 9-kd protein from peach was isolated and purified by analytical cation-exchange chromatography with an HPLC system (AKTA Purifier Pharmacia-Biotech, Uppsala, Sweden), introducing 1 mL of the raw extract after dilution to reach a correct pH and molarity into a Resource-S (1 mL) column (Pharmacia-Biotech). The mobile phase was sodium acetate 3-hydrate buffer A ($\text{CH}_3\text{COONa} \times 3 \text{H}_2\text{O}$ 50 mmol/L [pH 5]) and buffer B ($\text{CH}_3\text{COONa} \times 3 \text{H}_2\text{O}$ 50 mmol/L [pH 5] plus NaCl 1 mol/L). The gradient length was 20 column volumes. Absorbance was monitored at 280 nm, with a flow rate of 1 mL/min. After the peak corresponding to the unbound material, only 1 further peak was present on the chromatogram. Because this fraction, analyzed by SDS/PAGE, contained the 9-kd protein with other higher MW impurities, further resolution was achieved by gel filtration.

Gel filtration. Cationic exchange-concentrated fractions were separated on a Superdex 75 column equilibrated and eluted with 15 mmol/L NaCl in 50 mmol/L of sodium acetate trihydrate buffer (pH 5) at a flow rate of 1 mL/min. Before the first run, a calibration curve was prepared by measuring the elution volumes of various standard substances at known MWs: ribonuclease, 13.7 kd; chymotrypsinogen A, 25 kd; ovalbumin, 43 kd; and blue dextran 2000, 67 kd (Pharmacia Biotech). The sample injected was 0.5 mL, and absorbance was monitored at 280 nm. The chromatogram showed 2 peaks. The fractions corresponding to the peaks were concentrated, analyzed by SDS-PAGE, and stored at -20°C after measuring their protein content by the method of Warburg and Christian¹² and reading by spectrophotometer at 260 and 280 nm.

To confirm the purity of the 9-kd protein, separated in 2 chromatographic steps, we performed SDS-PAGE by using the method described.

Detection of IgE-binding capacity of the 9-kd peach protein by immunoblotting inhibition. In the immunoblotting inhibition experiment, aliquots of 500 μL samples of pooled sera from all patients were inhibited with 500 μL of the undiluted 9-kd purified fraction (0.3 mg of protein) and 500 μL of serial dilutions containing 0.03,

TABLE I. Characteristics of the patients included in the study

Patient	Age (y)	Sex	Peach			Birch		
			Symptoms	CAP (kU/L)	P-P	Symptoms	CAP (kU/L)	Other sensitizations
1	17	F	OAS	95.1	NT	No	<0.35	Prunoideae
2	21	F	OAS	14.5	NT	No	<0.35	Prunoideae
3	18	M	Urticaria, AE	56.4	++++	No	<0.35	Prunoideae, sesame seeds, almond, walnut, peanut
4	30	F	Urticaria, GE	27.8	NT	No	<0.35	Prunoideae, maize, almond, hazelnut, walnut, peanut
5	23	F	Urticaria, AE	13.0	++++	No	<0.35	Prunoideae, tomato, almond
6	36	F	OAS, GE	4.62	NT	No	<0.35	Prunoideae, bean
7	46	F	Urticaria, GE	1.2	++++	No	<0.35	Cherry, strawberry, banana, melon
8	35	F	OAS	1.25	++++	No	<0.35	
9	31	F	OAS	>100	NT	No	38.7	Prunoideae, hazelnut
10	33	F	OAS, urticaria, vomiting	64.8	NT	No	1.20	Prunoideae, hazelnut
11	52	F	OAS	2.04	++	Asthma	>100	
12	29	M	AE if touched	43.6	+++	No	62.8	
13	23	F	OAS	7.74	NT	RC	24.9	Kiwi, apple, hazelnut, peanut
14	17	F	OAS	3.4	NT	R	6.47	Prunoideae, maize, sunflower seeds
15	20	M	OAS	2.42	++++	No	14.2	Prunoideae, hazelnut

P-P, Prick-prick method; AE, angioedema; GE, glottic edema; RC, rhinoconjunctivitis; R, rhinitis; CAP, CAP System; NT, not tested.

0.003, 0.0003, 0.00003, and 0.000003 mg of purified protein. After 1 hour, strips of nitrocellulose with the electroblotted crude peach extract were incubated with the inhibited sera, as described before. The strips were then washed and incubated with I^{125} -labeled anti-human IgE for 6 hours and exposed to x-ray film at -70°C . Simultaneously, immunoblotting was done by using pooled sera from allergic patients to identify the IgE-binding pattern of total peach extract and the purity and allergenic activity of the 9-kd fraction. Immunoblotting of the total peach extract and the 9-kd fraction were also done by using pooled serum from healthy nonallergic patients.

Reduction and pyridylethylation. The 9-kd protein was dissolved in 450 μL of a solution containing 6 mol/L guanidinium chloride, 0.1 mol/L Tris, and 1 mmol/L EDTA (pH 8.3). Dithiothreitol was added to a final concentration of 2 mmol/L. The solution was incubated for 1 hour at 37°C in an oxygen-free N_2 atmosphere. One microliter of 4-vinyl pyridine (Merck) was added, and the solution was incubated overnight in the dark at room temperature.

Protein cleavage and trypsin digestion. A total of 250 μg of reduced and pyridylethylated protein was dissolved in 400 μL of 0.2 mol/L N-ethyl morpholine acetate (pH 8.3). The reaction was run with trypsin 1% (wt/wt, Promega) (stock solution 1 mg/mL in HCl 0.1 mmol/L) in a slow N_2 stream for 1 hour at 37°C . The procedure was repeated twice to raise the yield. The reaction was stopped by freezing the sample.

HPLC separation of the peptide mixture. Tryptic peptides were separated by reverse-phase HPLC on a C18 column (4.6 mm $\times$ 25 cm, Vydac), in 0.1% trifluoroacetic acid with a 2-step linear gradient of 0% to 40% acetonitrile in 1 hour and 40% to 70% acetonitrile in 10 minutes at a flow rate of 0.7 mL/min.

Amino acid sequencing. Protein and peptide sequence analysis was done on a Perkin-Elmer Applied Biosystems 470A gas-phase sequencer equipped with a 120A phenylthiohydantoin-amino acid derivative analyzer. All chemicals were from Perkin-Elmer Applied Biosystems.

Isoelectrofocusing. The 9-kd purified protein was focused by a

Pharmacia-LKB Phast System Ready by using Phast Gel, with a pH gradient from 3 to 9. The gel was fixed and stained with Coomassie Brilliant Blue R-250 and run according to the manufacturer's instructions.

Periodic acid-Schiff stain. Periodic acid-Schiff (PAS) staining was done to detect glycosylation of proteins. Six, 9, and 12 μL of purified 9-kd protein, corresponding to the protein content of 4.8, 7.2, and 9.6 μg , was run into mini-gel and blotted onto Problot membrane (Perkin-Elmer, Applied Biosystems), as described by Towbin and Gordon,¹³ at a constant 16 V for 60 minutes, with methanol and SDS omitted from the buffer. Two identical membranes were blotted at the same time, one stained with Coomassie R-250 and the other used for PAS staining for glycoproteins. For PAS staining, the membrane was fixed in 12% trichloroacetic acid for 1 hour and tested with a 7% acetic acid solution containing 2% KIO_4 and maintained at 4°C for 1 hour. The membrane was then immersed in Schiff's reagent and kept in the dark at 4°C overnight. The background was destained in methanol; glycoproteins appeared as purple bands. Milk whey proteins were used as controls.

RESULTS

Patients

Fifteen patients were recruited for the in vitro study. Their demographic data, specific IgE values (CAP System/RAST) and SPT responses for peach and birch, and other sensitizations are reported in Table I. Patients 1 to 8 were allergic to peach and/or other Prunoideae fruits but not to birch pollen, whereas patients 9 to 15 had positive IgE levels for birch pollen but only patients 11, 13, and 14 had clinical symptoms for birch. Patients 4, 6, and 7 were admitted on the basis of severe documented anaphylactic reactions to peach (glottic edema), whereas all other patients reported OAS symptoms to peach and were admitted after a positive oral challenge response.

The open challenge with fresh peach induced OAS symptoms in these 12 patients, 3 of whom also experienced generalized urticaria and 1 who experienced gastrointestinal symptoms, vomiting, and diarrhea in response to increasing amounts of the fruit. The provocative doses ranged from 4 to 64 g.

SDS-PAGE/immunoblotting

Coomassie Brilliant Blue–stained profiles of peach proteins showed different allergenic components with apparent MWs ranging from 9 to 80 kD. Fig 1 illustrates the IgE immunoblotting of peach extract in 15 patients, showing IgE binding at 9 kD in all of them and thus confirming that the 9-kD component is the major peach allergen. We found IgE binding only to the 9-kD component in the group with a negative CAP System result to birch, whereas in the group with a positive CAP System result to birch (mostly asymptomatic, with only 2 having symptoms in the birch pollen season), we identified several IgE-binding components at near 40, 48, 50, and 70 kD but not to the 20-kD protein, which probably corresponds to Bet v 1. Only 4 patients (9, 10, 12, and 14) showed IgE binding to a 15-kD allergen, which is likely to be a profilin.

HPLC

Fig 2, A, shows the chromatographic profile obtained from the cationic-exchange column. Besides the first peak, which corresponds to the unbound material, there was one more peak. Analysis of this second fraction by SDS-PAGE and silver staining (data not shown) showed that the 9-kD protein was not pure because other proteins at higher MWs were also present. This impure fraction, with a volume of 1 mL, had a protein content of 0.3 mg/mL. After 30 runs, we collected 30 mL, which was concentrated to a final volume of 2 mL with a protein content of 1.75 mg/mL. This concentrated fraction (0.5 mL) was injected onto a gel-filtration column, and the 2 peaks are shown in Fig 2, B. Comparison of the chromatographic profile with the markers led us to collect the fractions corresponding to the second peak, showing an appropriate MW. About 3.5 mL of this peak were collected at each run. The final volume of the purified 9-kD protein was 15 mL, with a protein content of 0.1 mg/mL. This was again concentrated to a final volume of 2.5 mL, with a protein content of 0.61 mg/mL and was analyzed in SDS-PAGE (Fig 3).

Immunoblotting and immunoblotting inhibition

The allergenicity of the purified protein was verified by immunoblotting with pooled sera from the patients under investigation. There was a strong IgE binding to the purified protein. No IgE binding was observed in healthy nonallergic patients for either peach crude extract or the 9-kD purified extract (data not shown).

To demonstrate that this protein was the same as the one in the crude extract, we tested the crude extract from the purified 9-kD protein in immunoblotting inhibition

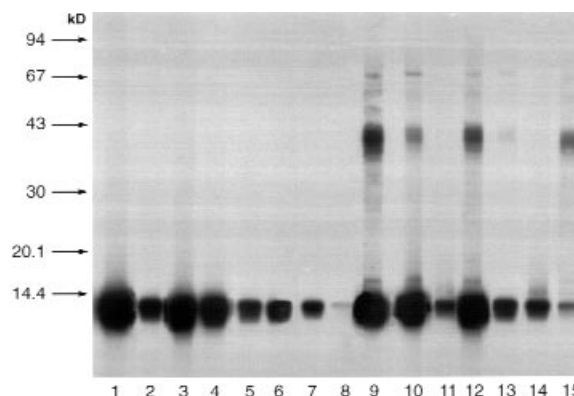


FIG 1. IgE immunoblot of peach extract with the sera from 15 peach-sensitive patients (patients 1 to 8 without specific IgE to birch pollen and patients 9 to 15 with specific IgE to birch pollen). The 9-kD protein is the major allergen (100% of binding).

experiments (Fig 4), obtaining complete inhibition of the 9-kD fraction of the crude extract even at the highest dilutions and no inhibition for all the other proteins with higher MWs. The lack of nonspecific binding of the 9-kD component was demonstrated with sera from subjects not allergic to peach with low and high levels of total IgE, with sera from 1 nonallergic individual, and with the buffer control (data not shown).

PAS stain

PAS staining for glycosylation on the 9-kD protein purified from peach and on a positive control excluded any glycosylation.

Isoelectrofocusing

The isoelectrofocusing experiment showed an estimated pI value greater than 9 for the 9-kD protein on the basis of the upper scale of the gradient. This was in agreement both with the theoretical value calculated from the amino acid composition of the protein (theoretical pI, 9.25) and with its cathodic mobility in native electrophoresis at pH 8.6.

Amino acid sequencing

The N-terminal sequence of the unmodified 9-kD protein and the sequence of some tryptic peptides obtained after pyridylethylation are reported in Table II. A database search showed a very high degree of homology (average, 65%), with several proteins belonging to the lipid transfer protein (LTP) family.

DISCUSSION

In this study we identified, purified, and characterized the major allergen from peach as a 9-kD protein with a high pI and no glycosylated residues. Amino acid sequencing indicated that the protein belongs to the LTP family. Its IgE-binding capacity, verified by repeating the immunoblotting with pooled sera from the patients, and

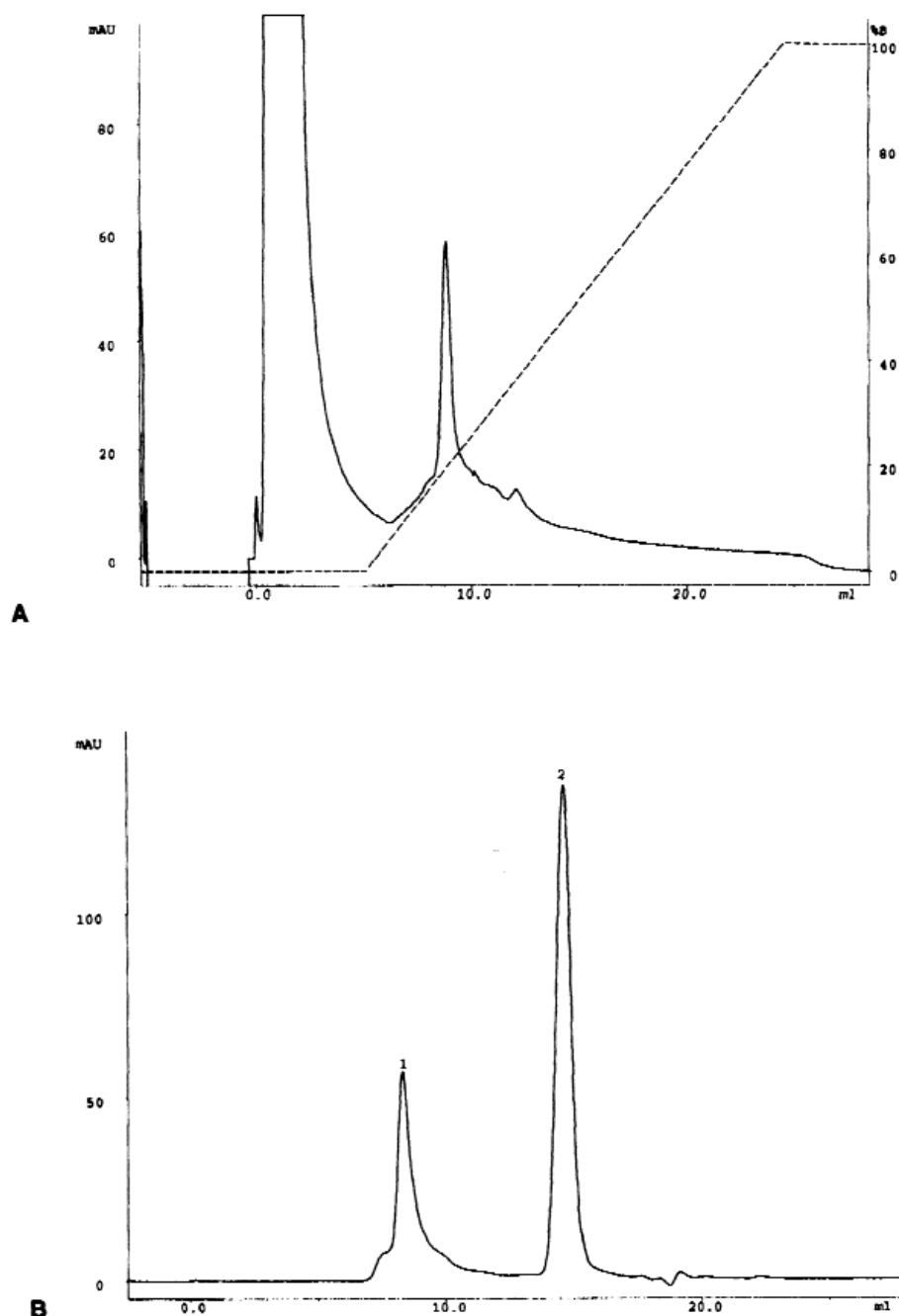


FIG 2. Cationic exchange of crude peach extract fractionated over the HPLC Resource-S column (**A**). Gel filtration of peak in cationic-exchange chromatography (**B**).

the complete inhibition of the blotting by preincubation with the purified 9-kd protein undoubtedly demonstrate that this is the major allergen from peach, and therefore it merits the denomination Pru p 1, which has already been added to the official list of allergens by the World Health Organization/International Union of Immunologic Societies Subcommittee for Allergen Nomenclature.

Sensitization to this allergen is independent of hypersensitivity to pollens because it was the only sensitization

found in subjects allergic to peach without pollinosis. The different pattern of IgE reactivity to peach allergens in patients with or without pollinosis emerged in our previous study on allergenicity of Prunoideae fruits,⁷ with the low MW allergen, so far not reported in the literature, recognized only by IgE antibodies from patients without pollinosis. This stresses the importance of selecting patients sensitized to a single allergen source with adequate clinical demonstration of their allergic symptoms

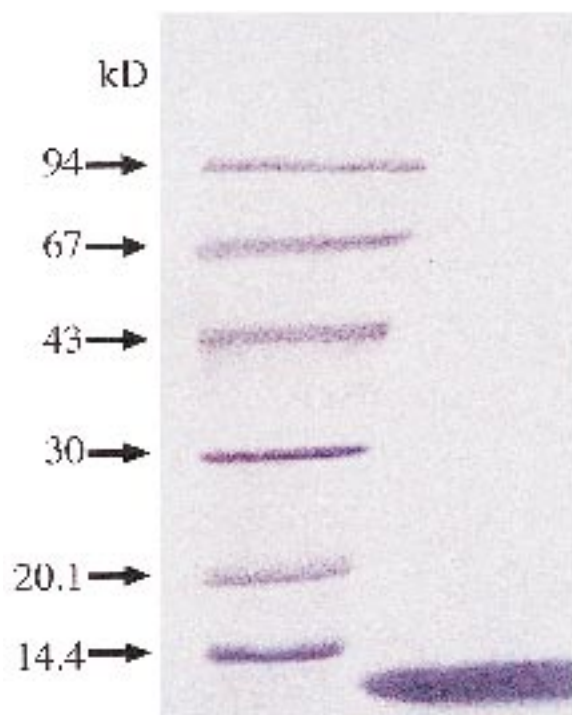


FIG 3. SDS-PAGE staining with Coomassie Brilliant Blue R-250 of the 9-kd purified protein.

TABLE II. N-terminal and tryptic peptides amino acid sequence of the 9-kd protein

N-terminal:	Ile-Thr-X-Gly-Gln-Val-Ser-Ala-Leu-Ala-Pro-X-Ile-Pro
TP 1:	Ile-Thr-Cys-Gly-Gln-Val-Ser-Ala-Leu-Ala-Pro-Cys-Ile-Pro-Tyr-Val-Arg
TP 2:	Gly-Gly-Gly-Ala-Val-Pro-Pro-Ala-Cys-Cys-Asn-Gly-Ile-Arg
TP 6:	Gln-Leu-Ser-Ala-Ser-Val-Pro-Gly-Val-Asn-Pro-Asn-Asn-Ala
TP 7:	Cys-Gly-Val-His-Ile-Pro-Tyr-Lys

for reliably evaluating the clinical role of the single allergens.

Studies on patients from Spain allergic to Rosaceae fruits, including peach, and to grass pollen confirmed our previous report that patients allergic only to Rosaceae fruits have a different IgE-reactivity pattern from those allergic to Rosaceae fruits and grass pollen. In the latter, cross-reacting molecules in the 2 allergen sources were found and identified as profilin and carbohydrate cross-reactive determinants,¹⁴ whereas other structures were involved in allergic reactions to Rosaceae fruits in patients without pollinosis.¹⁵

Our present findings definitely confirm that in allergy to peach the clinical role of allergens cross-reacting with pollens is of little importance and that the allergic symptoms are clearly related to sensitization to the 9-kd allergen (ie, the LTP), which is likely to occur through the oral route.

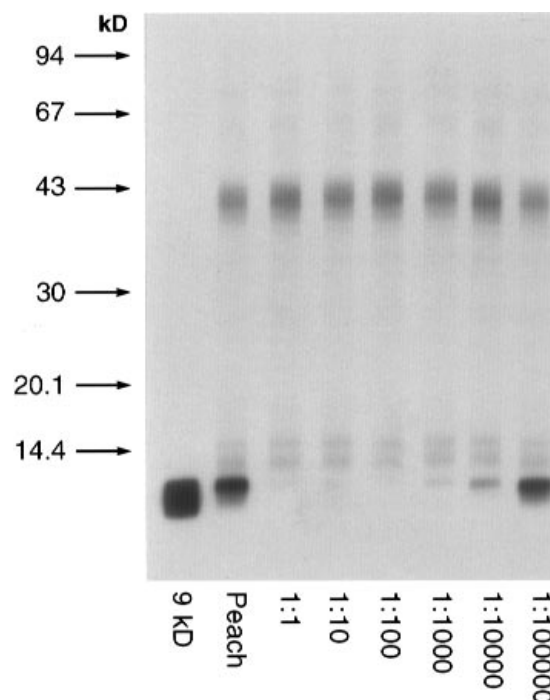


FIG 4. Inhibition of peach immunoblot by the 9-kd purified protein at different concentrations by using pooled sera from patients allergic to peach.

LTPs, named for their ability to transfer phospholipids from liposomes to mitochondria and first detected in spinach leaves,¹⁶ are functional molecules the biologic role of which is not completely known, but they are certainly involved in defensive processes of plants.¹⁷ LTPs contain 90 to 93 amino acids and present a homology of at least 30% in a number of plants, often with the highest level of expression in the leaves or in epidermal layers of cutinized organs.¹⁸ LTPs have antifungal and antibacterial properties, with their activity and potency depending on the different plants.^{19,20} The antibacterial activity is probably not plant specific, as demonstrated by the reduction of infection with *Pseudomonas syringae* in tobacco plants, to which LTP from barley was transgenically transferred.¹⁷

LTPs are part of the plant's defense system, which includes thionins, defensins, and hevein- and knottin-type antimicrobial peptides.¹⁷ These molecules are commonly located in the outer cell layer of the plant organs and become overexpressed in response to attack by pathogenic bacteria or fungi. Defensive molecules in plants have already been identified as important allergens, as demonstrated for pathogenesis-related proteins expressed under stress conditions, which include Bet v 1, Cor a 1, Mal d 1, Api g 1, and others.¹

The biologic properties of LTPs are currently under investigation, but little is known about their allergenicity in humans. From the search in the Swiss Prot Bank, we found that the LTP from peach has an amino acid sequence highly homologous with the LTPs from rice,

maize, tomato, and spinach, but the allergenic properties of these LTPs are unexplored. This allergen seems particularly important because it has never been described in peach, and in our hands it seemed to be one of the main proteins in this fruit. In the light of the antimicrobial activity of this family of molecules, this LTP may play some still unidentified protective role in the Prunoideae subfamily.

In the first demonstration we gave in 1994 of the allergenic role of a low MW allergen in peach, we found that this was the most important allergen in apricot, plum, and cherry, with complete cross-reactivity. This leads us to assume that it is also a very important cross-reacting allergen of the whole botanical subfamily in which it is likely to exert the same biologic function. These results cast new light on the question of panallergens because it seems that LTPs constitute a new class of allergenic proteins induced in difficult environmental conditions in plants and thus are probably very widespread in the vegetable kingdom. The close homologies in amino acid sequences among LTPs from several foods suggest the need for investigating clinical cross-reactivity to such foods. Analysis of IgE reactivity patterns against the peach extract showed another important allergen at high MW to which subjects allergic to inhalant allergens reacted. The sensitization to grass pollen in these patients suggests, also on the basis of the inhibition we observed in the previous study, that a similarly high MW allergen of grass pollen might be another important cross-reacting allergen.

In conclusion, this study identified the major allergen of peach as an LTP belonging to a group of molecules involved in plant defenses against microbial attacks. LTP from peach does not cross-react with any pollen allergen but is likely to cross-react with other LTPs because of the high homology in their amino acid sequences.

REFERENCES

- Pastorello EA, Incorvaia C, Pravettoni V, Ortolani C. Crossreactions in food allergy. *Clin Rev Allergy Immunol* 1997;15:415-27.
- Ebner C, Birkner T, Valenta R, Rumpold H, Breitenbach M, Scheiner O, et al. Common epitopes of birch pollen and apples—studies by Western and Northern blot. *J Allergy Clin Immunol* 1991;88:588-94.
- Ortolani C, Pastorello EA, Pravettoni V, Ispano M, Farioli L, Ansaloni R, et al. Fruit allergens. In: Oehling AK, Huerta-Lopez JG, editors. *Progress in Allergy and Clinical Immunology*. Seattle: Hogrefe & Huber; 1997. p. 65-8.
- Eriksson NE, Formgren H, Svenonius E. Food hypersensitivity in patients with pollen allergy. *Allergy* 1982;37:437-43.
- Ortolani C, Ispano M, Pastorello EA, Bigi A, Ansaloni R. The oral allergy syndrome. *Ann Allergy* 1988;61:47-52.
- Kivity S, Dunner K, Marian Y. The pattern of food hypersensitivity in patients with onset after 10 years of age. *Clin Exp Allergy* 1994;24:19-22.
- Pastorello EA, Ortolani C, Farioli L, Pravettoni V, Ispano M, Borga A, et al. Allergenic cross-reactivity among peach, apricot, plum and cherry in patients with oral allergy syndrome: an in vivo and in vitro study. *J Allergy Clin Immunol* 1994;94:699-707.
- Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch pollen allergy. *Allergy* 1983;38:167-72.
- Björkstén F, Halmepuro L, Hannuksela M, Lahti A. Extraction and properties of apple allergens. *Allergy* 1980;35:671-7.
- Lowry OH, Rosebrough NS, Fair AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;93:265-75.
- Neville DM. Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoresis in a discontinuous buffer system. *J Biol Chem* 1971;246:6328-63.
- Warburg H, Christian D. Isolierung und Kristallisation des Gärungsferments Enolase. *Biochem Z* 1941;310:384-91.
- Towbin H, Gordon J. Immunoblotting and do immunoblotting—current status and outlook. *J Immunol Methods* 1984;72:313-40.
- van Ree R, Fernandez-Rivas M, Cuevas M, van Wijngaarden M, Aalberse RC. Pollen-related allergy to peach and apple: an important role for profilin. *J Allergy Clin Immunol* 1995;95:726-34.
- Fernandez-Rivas M, van Ree R, Cuevas M. Allergy to Rosaceae fruits without related pollinosis. *J Allergy Clin Immunol* 1997;100:728-33.
- Kader JC, Julienne M, Vergnolle C. Purification and characterization of a spinach-leaf protein capable of transferring phospholipids from liposome to mitochondria or chloroplasts. *Eur J Biochem* 1984;139:411-6.
- Broekaert WF, Cammue BPA, De Bolle MFC, Thevissen K, De Samblanx GW, Osborn RW. Antimicrobial peptides from plants. *Crit Rev Plant Sci* 1997;16:297-323.
- Clark AM, Bohnert HJ. Epidermis-specific transcripts. Nucleotide sequence of a full-length cDNA of EPI12, encoding a putative lipid transfer protein. *Plant Physiol* 1993;103:677-8.
- Terras FRG, Goderis IJ, Van Leuven F, Vanderleyden J, Cammue BPA, Broekaert WF. In vitro antifungal activity of a radish (*Raphanus sativus*) seed protein homologous to nonspecific lipid transfer proteins. *Plant Physiol* 1992;100:1055-8.
- Molina A, Segura A, Garcia-Olmedo F. Lipid transfer proteins (LTPs) from barley and maize leaves are potent inhibitors of bacterial and fungal plant pathogens. *FEBS Lett* 1993;316:119-22.