

Lipid-transfer proteins as potential plant panallergens: cross-reactivity among proteins of *Artemisia* pollen, *Castanea* nut and Rosaceae fruits, with different IgE-binding capacities

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Summary

Background Lipid-transfer proteins (LTPs), but not Bet v 1 homologues, have been identified as major allergens of apple and peach in the Rosaceae fruit-allergic population in the Mediterranean area. Many of these patients show cosensitization to mugwort pollen. LTPs have an ubiquitous distribution in tissues of many plant species, and have been proposed as a novel type of plant panallergens.

Objective We sought to isolate LTPs from *Artemisia* pollen and from a plant food not belonging to the Rosaceae family, such as chestnut nut, and to compare their amino acid sequences and IgE-binding capacities with those of apple and peach LTPs.

Methods Allergens (LTPs) were isolated by different chromatographic methods (gel-filtration, ion exchange and/or reverse-phase HPLC), and characterized by N-terminal amino acid sequencing and MALDI analysis. Specific IgE-quantification and immunodetection, as well as immunoblot and ELISA inhibition assays, were carried out using sera from patients allergic to both apple and peach.

Results Purified LTPs from *Artemisia* pollen and from chestnut seed showed molecular masses about 9 700d, and 43–50% sequence identity with the equivalent allergens of apple and peach in the first 30 N-terminal residues, which comprise about one third of the total amino acid sequence. A similar degree of sequence identity (50%) was found between the *Artemisia* and chestnut proteins. Both isolated LTPs bound specific IgE of sera from Rosaceae fruits allergic patients. However, substantially lower values of specific IgE-binding and maximum ELISA inhibition percentages were obtained for *Artemisia* and chestnut LTPs when compared to those from apple and peach.

Conclusion LTPs from *Artemisia* pollen and chestnut crossreact with allergens (LTPs) of Rosaceae fruits, but significant differences in specific IgE-binding capacities were observed among members of the plant LTP family. Thus, further studies are needed to evaluate the clinical significance of the observed cross-reactivities of plant LTPs.

Keywords: Lipid-transfer proteins, plant panallergens, Rosaceae fruits, *Artemisia* pollen, chestnut seed, cross-reactions.

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Introduction

Allergy to fruits is increasingly associated with pollinosis or with latex sensitization (latex–fruit syndrome [1–4]).

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Adverse reactions to Rosaceae fruits seem to be particularly prominent in this context [3]. Allergy to these fruits (apple, peach, pear, cherry, etc.) is often linked to birch pollinosis in Central and Northern Europe and in North America [1,5]. This association has been explained by cross-reactivity of IgE antibodies to allergens which are present in both pollen and fruits, mainly Bet v 1 (the major birch pollen allergen) and its homologues identified in several Rosaceae fruits [6,7]. Other allergens, such as profilins and isoflavone reductases [8,9], also seem to be involved in the pollen-fruit cross-reactions.

A different pattern of sensitization to Rosaceae fruits is found in populations living in areas virtually free of birch and other *Fagales* trees, as is the case in several Mediterranean areas [10–12]. Most patients in these populations show undetectable specific IgE levels to Bet v 1. Several of them have associated grass pollinosis, profilin and carbohydrate cross-reactive determinants being two prominent cross-sensitization agents [11]. A second group of patients belonging to the birch-free population are allergic to Rosaceae fruits without related grass pollinosis [10,12]. Interestingly, *in vitro* cross-reactions and specific IgE to mugwort, but not to ryegrass and birch pollen, have been reported in several members of this group [12,13], thus suggesting a potential link between mugwort pollen and Rosaceae fruit allergy in, at least, the Spanish area.

Lipid-transfer proteins (LTPs) have recently been identified as major apple and peach allergens in patients allergic to Rosaceae fruits, but not sensitized to birch pollen [14,15]. The isolated allergens are 9 kd proteins, which belong to a protein family widely distributed throughout the plant kingdom [16]. Typical members of the LTP family are polypeptides comprising 91–95 amino acids, with eight conserved cysteines forming four disulphide bridges, and showing different degrees of sequence identity among them [16]. LTPs usually accumulate in the outer epidermal layers of plant organs [16,17], thus explaining the stronger allergenicity of peels with respect to pulps of Rosaceae fruits [18].

The wide distribution of LTPs among plant foods suggests a potential role of these polypeptides as plant panallergens, assumed to be responsible for cross-reactions among fruits, nuts, and/or pollens. However, the information about this matter is still very limited. Besides the apple and peach allergens, only a LTP from *Parietaria* pollen [19] has been implicated in allergy. Additionally, low molecular size allergens from *Brassica rapa* [20] and soybean [21] are weakly related to the LTP family, though their actual structural and allergenic relationship remains to be investigated. Thus, a joint study of the sequence similarities and IgE-binding properties of LTPs from different sources seems to be warranted. We report here such a study, comparing purified LTPs from mugwort pollen and chestnut

seed with those from apple and peach. The mugwort protein was chosen based on the cross-reactions between Rosaceae fruits and *Artemisia* pollen mentioned above, and that from chestnut because it is botanically distant from apple and peach (*Castanea sativa* belongs to the Fagaceae family), and due to the sensitization to this nut observed in fruit allergic patients [12,13].

Materials and methods

Sera from allergic patients

A pool of six sera from patients allergic to apple and peach were used in this study. All patients showed immediate hypersensitivity to both fruits according to the clinical history, positive skin prick tests (SPT) with the corresponding fruit extracts, and specific IgE levels to both fruits above 4.1 kU/L. Additionally, the six patients had positive SPT responses to the mugwort pollen extract and specific IgE values greater than 1.0 kU/L to this pollen, whereas SPT and specific IgE determination were negative in all cases when birch pollen was assayed.

Pooled sera from three subjects not sensitized to fruit (negative SPT and undetectable specific IgE to apple, peach and plum), but with total serum IgE levels similar to those of the fruit allergic patients, were tested as negative control.

Total and specific IgE levels were determined by the Pharmacia CAP system FEIA (Pharmacia Diagnostics, Uppsala, Sweden). SPTs were performed with commercial glycerinated preparations (ALK-Abelló, Madrid, Spain) using conventional procedures, and weal areas 7 mm²-greater than that produced by the saline control were considered positive.

Crude extracts preparation

Ground skins of apple (*Malus domestica*) and peach (*Prunus persica*), mugwort (*Artemisia vulgaris*) pollen, and ground hulled chestnuts (*Castanea sativa*), were defatted with acetone (1 : 5 [w/v]; 1 h; 4 °C), and then with ethanol:ether 1 : 3 [v/v] (1 : 5 [w/v]; 1 h; 4 °C). The dried material was then extracted with PBS buffer (0.1 M sodium phosphate, pH 7.0, 0.15 M NaCl; 1 : 5 [w/v]; 1 h; 4 °C). After centrifugation (121 000 g; 25 min; 4 °C), the supernatant was dialysed against H₂O and freeze-dried.

Protein concentration was determined according to Bradford [22].

Purification and characterization of LTPs (allergens)

Apple and peach allergens were isolated by reverse-phase HPLC (RP-HPLC) using the same chromatographic conditions described by Sanchez-Monge *et al.* [14].

LTP from mugwort pollen was purified from a Tris-HCl extract (0.1 M Tris, pH 7.5, 10 mM EDTA; 1:5 [w/v]; 1 h; 4 °C) of defatted pollen. After centrifugation, dialysis against H₂O and freeze-drying, the extract was fractionated by RP-HPLC on a Vydac-C4 column (22 × 250 mm; particle size 10 µm; The Separations Group, Hesperia, CA, USA). Elution was performed with a two-step linear gradient of 2-propanol in 0.1% trifluoroacetic acid (0% to 30% in 120 min; 30% to 50% in 30 min and 80 min at 50%; 1 mL/min). Fractions containing the LTP were pooled, freeze-dried, and further rechromatographed on a semipreparative RP-HPLC Nucleosil 300-C4 column (8 × 250 mm; particle size 5 µm; Sugelabor, Madrid, Spain), eluting with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid (0% to 80% in 150 min; 0.5 mL/min).

Chestnut LTP was isolated from the corresponding PBS extract by an initial gel-filtration chromatographic fractionation on a Sephacryl S200 column (2.5 × 85 cm; Pharmacia Biotech, Uppsala, Sweden), using 0.1 M ammonium acetate as elution buffer (1 mL/min). Fractions enriched in chestnut LTP were pooled, freeze-dried, and then separated by RP-HPLC on a preparative Vydac-C4 column (22 × 250 mm; particle size 10 µm), eluting with a three-step linear gradient of acetonitrile in 0.1% trifluoroacetic acid (10% during 45 min; 10% to 35% in 140 min; 35% to 50% in 100 min and 50% to 85% in 15 min; 1 mL/min).

Peaks containing the corresponding allergen in each chromatographic separation were identified by SDS-PAGE and immunodetection. The isolated proteins were quantified by the bicinchoninic acid test [23].

SDS-PAGE was carried out on 4% to 20% precast polyacrylamide gels (Bio-Rad), following the technique described by Laemmli [24], except that reducing agents and heat treatment of samples were omitted.

N-terminal amino acid sequences were obtained by standard methods using an Applied Biosystems 477 A gas-phase sequencer.

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry analysis of purified LTPs was performed with a MALDI-2 spectrometer with delayed extraction (Kratos Analytical, Manchester, UK).

Immunodetection and immunoblot inhibition tests

Samples were fractionated by SDS-PAGE and then electrotransferred onto nitrocellulose membranes as in Towbin *et al.* [25]. Immunodetection with the pool of sera from fruit allergic patients or with control sera (1:10 dilutions) was conducted as previously described [14], using mouse antihuman IgE monoclonal antibody, rabbit antimouse IgG peroxidase-conjugated antibody, and the enhanced chemiluminescence method (Amersham, UK) for detection of IgE-binding components.

The isolated LTPs were used as inhibitors in immunoblot inhibition tests. The PBS extract from peach (15 µg of protein) was separated by SDS-PAGE and electrotransferred onto nitrocellulose membranes. The serum pool (1:10 dilution) was pre-incubated with each inhibitor (5 µg of purified allergen or bovine serum albumin as negative control, in a final volume of 1 mL) for 3 h at room temperature before exposure to the membranes.

Determination of specific IgE to purified LTPs

Polystyrene 96-well microtitre plates (Costar 3590, Corning, NY, USA) were coated with 50 µL of purified LTP at 20 µg/mL in PBS buffer, during 1 h at 37 °C. The coated wells were blocked with 1% bovine serum albumin in PBS buffer, and then incubated with 50 µL of the serum pool from fruit allergic patients (1:6 dilution) for 3 h at room temperature. After washing with 0.1% Tween-20 in PBS buffer, wells were incubated with a peroxidase-labelled antihuman IgE (Dako A/S, Denmark, Code P0259) for 1 h at room temperature. Plates were washed again, and then developed by incubating with 50 µL of peroxidase substrate buffer (0.012% H₂O₂, 0.66 mg/mL o-phenylenediamine, OPD; Dako, Code S2045). The reaction was stopped after 30 min with 50 µL of 4N H₂SO₄, and the OD was measured at 492 nm. Assays were performed in duplicate. A negative control (dilution buffer with 1% bovine serum albumin) was tested for each solid phase LTP. Additionally, serum from a patient not allergic to Rosaceae fruits was proved with all four LTPs, showing OD under 0.05 units in all cases.

Results were expressed in RAST Units (RU)/mL, using a control curve obtained with *Lolium perenne* extract on solid phase and a pool of sera from grass allergic patients previously calibrated against Phadebas RAST® (Pharmacia, Uppsala, Sweden).

ELISA-inhibition assays

Polystyrene 96-well microtitre plates were coated with 50 µL of isolated LTP solution (20 µg/mL) in PBS buffer for 1 h at 37 °C. After blocking with 1% bovine serum albumin in PBS buffer, wells were incubated with 25 µL of the serum pool (1:3 dilution) and 25 µL of serial dilutions of each purified LTP (range: 10–2 µg/mL) for 3 h at room temperature. After washing, wells were incubated with peroxidase-labelled antihuman IgE, and developed with OPD solution as indicated above.

The inhibition (%) of IgE-binding was determined as:

$$\text{inhibition (\%)} = 100 \times (1 - [(OD - OD_{100\%}) / (OD_{0\%} - OD_{100\%})])$$

OD is the result of test sera with LTP inhibitor, OD_{0%} is

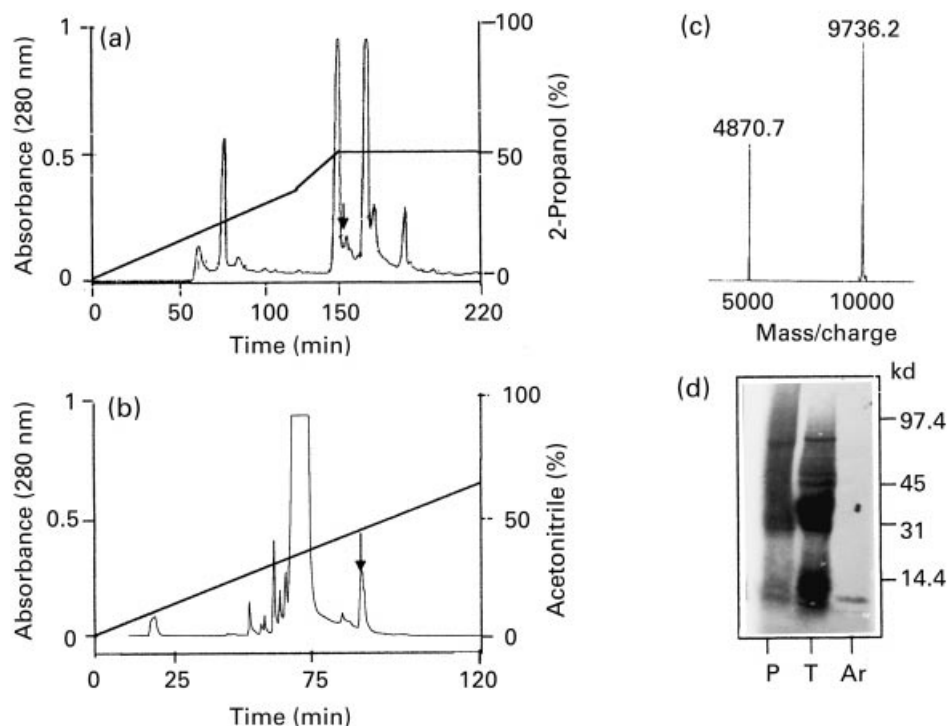


Fig. 1. (a) RP-HPLC fractionation on a preparative Vydac-C4 column of a Tris-HCl extract from defatted mugwort pollen. Arrow points to the peak containing the 12 kDa polypeptide. (b) RP-HPLC separation on a semipreparative Nucleosil 300-C4 column of the protein fractions indicated by an arrow in panel (a). Arrow points to the position of the purified protein. (c) MALDI analysis of the isolated mugwort pollen protein. The lower peak corresponds to the doubly charged ion, showing one-half of the actual molecular mass. (d) SDS-PAGE separation of PBS extract (P) and Tris-HCl extract (T) from mugwort pollen, and of the purified protein (Ar).

the test sera without LTP inhibitor, and $OD_{100\%}$ is LTP inhibitor in the absence of sera. Bovine serum albumin (negative control) was used to determine $OD_{0\%}$.

Results

The LTPs (allergens) of apple and peach were isolated from the corresponding peel extracts using the same protocol and RP-HPLC conditions previously reported by us [14]. The purity of the isolated proteins was determined by SDS-PAGE and MALDI analysis (not shown), obtaining similar results to those already published [14].

To purify the putative homologous polypeptide from mugwort pollen, a Tris-HCl extract was initially fractionated by RP-HPLC (Fig. 1a). Fractions containing the putative polypeptide (together with additional proteins) were further chromatographed on a different column (Fig. 1b). The peak corresponding to the low molecular weight component, as well as the homogeneity of the isolated protein, was checked by both SDS-PAGE and MALDI analysis. The purified polypeptide showed two sharp peaks with molecular masses of 9736 d and 4870 d

in the MALDI spectrum (Fig. 1c), and a single electrophoretic band with an apparent molecular weight of around 12 kD (Fig. 1d). The lower peak in the MALDI spectrum corresponds to the doubly charged ion, showing one-half of the actual molecular mass.

The putative chestnut allergen was isolated by gel-filtration chromatography of a PBS extract (Fig. 2a), followed by RP-HPLC separation of those gel-filtration fractions enriched in the chestnut low molecular weight band (Fig. 2b). The peaks including the putative allergen in both chromatographic separations were identified by SDS-PAGE and MALDI analysis. The isolated protein presented a major peak with a molecular mass of 9.725 d in the MALDI spectrum (Fig. 2c), and a single band of around 13 kD in SDS-PAGE.

Figure 3 shows the N-terminal amino acid sequences (more than one third of their complete sequence) of both mugwort and chestnut purified proteins compared to the known sequences of other allergen LTPs. No heterogeneity was found at any sequenced residue, thus confirming the purity of the isolated *Artemisia* and *Castanea* potential allergens. The primary structures determined clearly allow the inclusion of both purified proteins in the plant LTP

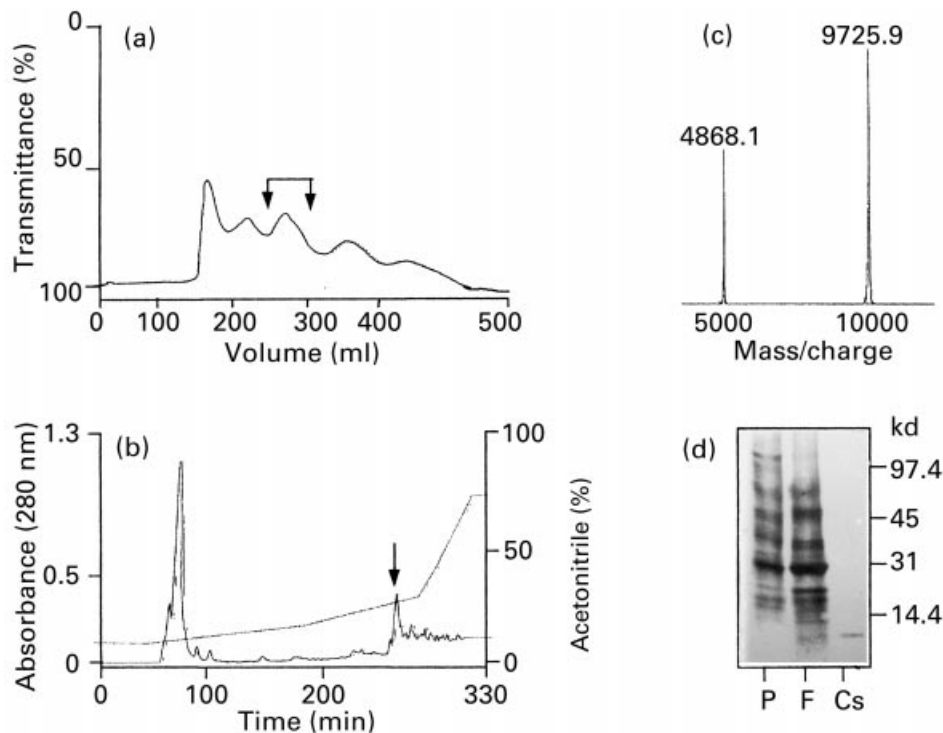


Fig. 2. (a) Gel-filtration on a Sephacryl S200 column of a PBS extract from chestnut seeds. Fractions enriched in the 13 kDa polypeptide are indicated. (b) RP-HPLC fractionation on a preparative Vydac-C4 column of the gel-filtration fractions indicated in panel (a). Arrow points to the position of the isolated protein. (c) MALDI analysis of the purified chestnut protein. The lower peak corresponds to the doubly charged ion, showing one-half of the actual molecular mass. (d) SDS-PAGE separation of PBS extract from chestnut seed (P), the gel-filtration fractions indicated in panel (a) (F), and the purified protein (Cs).

family. However, in contrast with the high degree of sequence identity between the apple and peach allergens (>90%), and between these and an almond member of this allergen family (>85%), a substantially lower degree of similarity was found between the homologous proteins of mugwort pollen and chestnut (50%), as well as between these and the Rosaceae fruit allergens (43–53% identity). The Parietaria pollen allergen is even weakly related, with values of sequence identity below 40% when compared with the other LTP sequences shown in Fig. 3.

The IgE-binding capacity of the *Artemisia* and *Castanea* proteins was assayed and compared with that of apple and peach allergens by IgE immunodetection using the serum pool from fruit allergic patients (Fig. 4a). A reactive band of around 12–13 kD was found in the all corresponding PBS extracts, and a positive recognition was shown by the isolated LTPs from mugwort and chestnut. Interestingly, a clearly stronger response, with respect to that of Rosaceae fruit allergens, was obtained with the *Castanea* protein. However, immunoblot inhibition tests using the isolated LTPs as inhibitors of the IgE-binding to the peach PBS extract (Fig. 4b) showed a full inhibition by the peach allergen, but only weak effects when testing the mugwort

and chestnut LTPs. No reaction was found for any of the purified proteins when negative control sera from patients not sensitized to fruits were tested.

To confirm the results of the immunodetection and immunoblot inhibition experiments, both quantification of specific IgE to the purified allergens and inhibition capacity in ELISA, using the pool of sera from fruit allergic patients, were performed. The specific IgE levels to the four isolated LTPs are listed in Table 1. The highest value found was to the peach allergen, followed by that to the apple allergen (60% of the peach LTP level), and those to mugwort and chestnut LTPs (around 25% with respect to the IgE to peach protein). Table 2 shows the results of the ELISA inhibition assay with the purified allergens both on the solid phase and as inhibitors. Each purified LTP showed an almost full inhibition by itself (positive control). Very high percentages of inhibition were also observed for the peach (>90%) and apple (>80%) proteins as inhibitors of IgE binding to the other three LTPs. On the contrary, the inhibition exerted by the mugwort and chestnut LTPs was clearly lower, both against each other (63–59%), and towards the peach (46–41%) and apple (61–40%) allergens.

(a)

LTP (allergen)	N-terminal amino acid sequence
	1 15 30 39
Ar	ALTCSDVSNKISPCLSYLKQ-GGEVP-ADCCAGVKGLND
Cs	SITCTQVSKSLMPCLTYLKSNGGSPPPGTCCQGVKL
Ma	ITCGQVTSSLAPCIGYVRS-GGAVPPA-CCNGI
Pr	ITCGQVSSSLAPCIPYVRG-GGAVPPA-CCNGIRNVNN
Al	ITCGQVSSNLAPCIPYVRG-GGAVPPA-CCNGIRNVNN
Par j	EETCGTVVRALMPCLPFVQG-KEKEPSKGCCSGAKRLDG

(b)

	Identity (%)					
	Ar	Cs	Ma	Pr	Al	Par
Ar	-	50	44	43	43	33
Cs		-	53	50	50	36
Ma			-	91	88	38
Pr				-	97	38
Al					-	38
Par						-

Fig. 3. (a) N-terminal amino acid sequences of the LTPs isolated from mugwort pollen (Ar) and chestnut seed (Cs), aligned with those previously reported from apple (Ma; Sanchez-Monge *et al.* [14]), peach (Pr, Sanchez-Monge *et al.* [14], and Pastorello *et al.* unpublished, Swiss-Prot accession number P81402), almond (Al, M. Suelves, unpublished, EMBL accession number X96714) and *Parietaria judaica* (Par j; Duro *et al.* [19]). Gaps (–) to obtain the proper sequence alignment are indicated. (b) Percentages of sequence identity among the six members of the allergen (LTP) family. Values have been determined according to the partial N-terminal sequences included in panel (a).

Discussion

The identification of LTPs as major apple and peach allergens [14,15] in birch pollen-free areas, together with the ubiquitous distribution of the plant LTP family [16], has led to propose this protein class as novel plant panallergens

[14]. In fact, besides the isolated apple and peach allergens (Pru p 3 and Mal d 3) and that from *Parietaria* pollen (Par j 1) [19], other still uncharacterized low molecular weight IgE-binding proteins from other Rosaceae fruits [10,26], as well as of distant phylogenetic plant species such as cereals [27], are probably members of the LTP group.

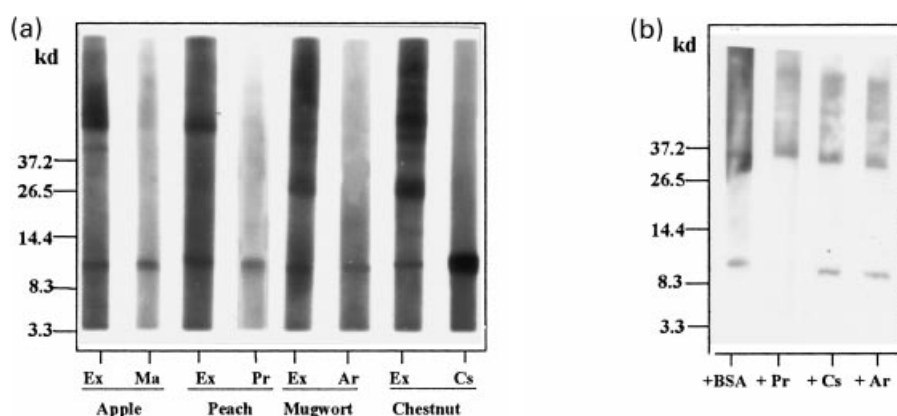


Fig. 4. (a) IgE immunodetection of PBS extracts (Ex; 15 μ g of protein) and purified LTP allergens (Ma, Pr, Ar and Cs; 5 μ g of protein) from apple, peach, mugwort and chestnut, respectively. Samples were separated by SDS-PAGE, electrotransferred onto nitrocellulose membranes, and immunodetected with the pool of sera from fruit allergic patients. (b) Immunoblot inhibition assays with purified LTPs from peach (+Pr), chestnut (+Cs) and mugwort (+Ar) as inhibitors. Bovine serum albumin (+BSA) was assayed as negative control. PBS extract from peach (15 μ g of protein) was separated by SDS-PAGE, electrotransferred onto nitrocellulose membranes, and immunodetected with the serum pool preincubated with 5 μ g of each inhibitor.

Table 1. Specific IgE to purified allergens

LTP on solid phase	OD*	RU/mL†
Peach	1.46	73.4
Apple	0.95	44.7
Chestnut	0.40	18.0
Mugwort	0.37	17.0

*Optical density at 492 nm obtained in the assay. Values are means ($n = 3$). †Result expressed in RAST Units/mL using a calibrated control curve and taking into account the dilution of the serum pool (sixfold).

Moreover, their tissue-specific accumulation, regulated developmental expression, and induction by biotic stress [16,17], suggest that substantial changes in allergen concentration can be expected depending on the plant tissue (i.e. peel and pulp of fruits) and on environmental, harvesting and storage conditions.

However, the experimental evidence in this field is still very scarce and it seems necessary to demonstrate the presence of LTPs in different plant foods and pollens, together with the characterization of their structural and allergenic properties. The results presented above on mugwort pollen and chestnut seed LTPs, both plant materials partially associated with Rosaceae fruit allergy [12,13], should be analysed in this context.

Lipid-transfer proteins from *A. vulgaris* pollen and *C. sativa* nut extracts were isolated using different chromatographic methods (Figs 1 and 2). The purified polypeptides are slightly greater (9726 and 9736 d) than those previously described from apple and peach (9058 and 9138 d) [14] as determined by MALDI analysis. They show higher apparent molecular weights in SDS-PAGE (around 12–13 kd), as is

also the case for the isolated proteins from Rosaceae fruits [14]. Their N-terminal amino acid sequences confirm their inclusion in the plant LTP family. However, whereas the members from Rosaceae species (peach, apple and almond) show closely related primary structures (88–97% of sequence identity), the purified mugwort and chestnut LTPs are clearly less similar to the Rosaceae allergens, and to each other (53–43% of sequence identity). The allergen of *Parietaria* pollen, Par j 1, seems to be even more structurally distant (see Fig. 3). Interestingly, low molecular size allergens weakly related to the LTP family, but conserving the positions of most cysteines and some residues adjacent to them, have been described in *Brassica* pollen and soybean hull [20,21].

A serum pool from fruit allergic patients that recognizes the apple and peach LTPs also contains specific IgE to the expected low molecular weight bands of the mugwort and chestnut extracts, as well as to the corresponding isolated LTPs (Fig. 4). Surprisingly, the *Castanea* polypeptide shows an even stronger reaction than the apple and peach allergens. The structural explanation for this result (confirmed by testing two different batches of the isolated protein) remains unknown. In contrast with these immunodetection data, a distinctive behaviour was shown by the purified LTPs in immunoblot inhibition assays. Full inhibition of the IgE-binding to the corresponding low molecular size band of the peach extract was obtained when the peach allergen was assayed as inhibitor, whereas the mugwort and chestnut LTPs exerted no apparent effect. It had been previously demonstrated that both isolated apple and peach LTPs inhibited the recognition by IgE of the 13-kd component of the crude extract from both fruits [14]. Thus, it seems that the mugwort and chestnut proteins share some IgE epitopes with the apple and peach allergens, but lack other main ones which are present in the fruit LTPs. Two complementary lines of evidence support this hypothesis. Quantification of the specific IgE levels to the four purified LTPs shows higher values for the apple and peach allergens than those for mugwort and chestnut (one-fourth of the IgE level to peach LTP). Furthermore, ELISA inhibition assays reveal almost full inhibition of the IgE-binding by all four purified proteins when the apple and peach LTPs are used as inhibitors, whereas the IgE-binding by the fruit LTPs is only partially inhibited by those from mugwort and chestnut.

Joint consideration of the data discussed above indicates a partial cross-reaction between Rosaceae LTPs and those from different plant sources. The association and *in vitro* cross-reactions between mugwort pollen and Rosaceae fruit allergy previously reported [12,13], as well as the susceptibility of fruit allergic patients to chestnut [12,13], can be explained, at least in part, by the common epitopes shared by LTPs from these foods and pollen. The isolation of new members of this

Table 2. ELISA inhibition assay with purified allergens

LTP on solid phase	Maximum inhibition (%) of IgE binding*			
	Inhibitor LTP			
	Peach	Apple	Mugwort	Chestnut
Peach	96.4	83.2	46.3	41.2
Apple	96.7	97.8	61.3	40.3
Mugwort	90.4	92.3	99.3	63.1
Chestnut	91.1	92.8	58.8	85.4

*Mean values ($n = 2$). The percentage inhibition was calculated as indicated in the Materials and methods section.

protein family from other Rosaceae fruits, and other botanically distant plant foods and pollens, could clarify the clinical importance of LTPs as plant panallergens.

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