

REVIEW

Plant non-specific lipid transfer proteins as food and pollen allergens

G. Salcedo*, R. Sanchez-Monge*, A. Diaz-Perales*, G. Garcia-Casado* and D. Barber†

*Unidad de Bioquímica, Departamento de Biotecnología, ETS Ingenieros Agrónomos and †Departamento I+D, ALK-Abelló, Madrid, Spain

Summary

Several members of the plant non-specific lipid transfer protein (LTP) family have been identified as relevant allergens in foods and pollens. These allergens are highly resistant to both heat treatment and proteolytic digestion. These characteristics have been related with the induction of severe systemic reactions in many patients, and with the possibility of being primary sensitizers by the oral route. A specific geographical distribution pattern of sensitization to LTP allergens has been uncovered. This allergen family is particularly important in the Mediterranean area, but shows a very limited incidence in Central and Northern Europe. The potential role in the plant, as well as the biochemical and allergenic properties of the LTP family, are reviewed here.

Introduction

Pollen-related food allergy is the most common food hypersensitivity in the adult population [1, 2]. In particular, *Fagales* (birch) pollen-related allergy to *Rosaceae* fruits (mainly apple), vegetables, such as celery and carrot, and hazelnut, affects many patients in Central and Northern Europe, where birch trees are abundant [2–4]. The presence of homologous allergens in pollens and fruit/vegetables, mainly members of the Bet v 1 (the major birch pollen allergen) and profilin families, as well as cross-reactive carbohydrate determinants, seems to be responsible for such cross-sensitizations [3, 5, 6]. Patients suffering this type of food allergy usually present mild symptoms restricted to the oral cavity (oral allergy syndrome, OAS), and the pollen allergens, but not the food allergens, are those causing the primary sensitization [1, 3]. However, systemic symptoms ranging from urticaria to anaphylactic reactions have been described in a significant percentage of patients with pollen-related food allergy [7, 8]. Interestingly, both profilins and Bet v 1 homologues seem to be labile allergens, readily hydrolysed by simulated gastric fluid [5, 9, 10].

In contrast to the above-described scenario, the population allergic to *Rosaceae* fruits (especially peach) in the Mediterranean area presents a different clinical and sensitization pattern. A relevant amount of patients (>20%) show fruit allergy without related pollinosis [11, 12] and, accordingly, specific IgE to Bet v 1 is hardly detected (<10%; see ref. [12]). Furthermore, systemic reactions are frequently observed (20–30%), even in the absence of OAS [12, 13]. Non-specific lipid transfer proteins have been identified as the major allergens in this population, not only those of *Rosaceae* fruits [14–17], but also those of other plant foods [18–21]. The high resistance of lipid transfer proteins (LTPs) to proteolytic digestion and heat treatment [9, 22, 23] points to these allergens as primary

sensitizers by ingestion, and has been related with the severe reactions induced in many patients. All these characteristics have led to the proposal of LTPs as model allergens of true food allergy in a brief, but excellent, recent paper by Van Ree [24]. Besides, relevant allergens from *Parietaria*, *Artemisia* and *Olea* pollen are also members of the LTP family [25–28].

Plant LTPs: general features and potential functions

Plant LTPs form a family of basic polypeptides ($pI \sim 9$) with 91–95 amino acid residues in their primary structure and a molecular size of approximately 9 kDa [29, 30]. Different degrees of sequence identity (from 30% to 95%) are found between members of the family from different species. However, eight conserved cysteines forming four disulphide bridges, which are responsible for the LTP compact folding, are present in all members [29, 31].

The experimentally determined 3-dimensional (3-D) structure of cereal LTPs has led some to propose an all- α -type compact structure as the LTP fold [31]. This fold includes 4 α -helices separated by short turns and a flexible and non-structured C-terminal coil (Fig. 1). This 3-D structure is firmly held by the four disulphide bridges mentioned above, and presents a large internal hydrophobic tunnel-like cavity running through the long axis of the molecule. The cavity can harbour lipidic components (i.e. acyl tails of fatty acids and phospholipids) [29, 31]. Interestingly, other plant allergens, such as soybean Gly m 1, the α -amylase/trypsin inhibitors from cereal flours and some 2S storage proteins, show 3-D structures close to the LTP fold [31].

Different genetic variants (isoforms) encoded by a multi-gene family (up to 15 genes in *A. thaliana*) [32] have been detected in most plant species studied. Although differential tissue and developmental expression patterns have been described for many isoforms (i.e. in fruit peach; see ref. [33]), most LTPs are mainly localized in the aerial plant organs (leaves, seeds, flowers, fruits), showing low or even no expression in roots [29, 33]. These allergens are preferentially

Correspondence: Gabriel Salcedo, Unidad de Bioquímica, Departamento de Biotecnología, ETS Ingenieros Agrónomos, 28040 Madrid, Spain.
E-mail: gsalcedo@bit.etsia.upm.es

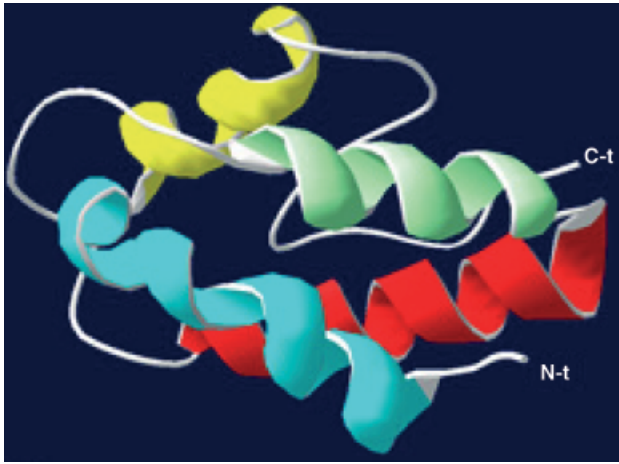


Fig. 1. Three-dimensional (3-D) model of the major peach allergen Pru p 3. The four α -helices of the lipid transfer protein (LTP) fold are shown in different colours.

accumulated in the outer epidermal cell layers of the exposed plant surfaces, such as fruit peels [34]. Therefore, it has been demonstrated that peels of *Rosaceae* fruits have a higher allergenic potency than pulps [35].

LTPs were originally named as such because of their lipid binding properties and their *in vitro* capacity to transfer a broad range of lipids between membranes [29]. This hypothetical role in the intracellular trafficking of lipids is not consistent with the extracellular (cell wall-associated) location of LTPs [34]. In contrast, the strongest experimental evidence now available points to a role in plant defence mechanisms against pathogens (bacteria and fungi) [34]. The bactericidal and/or fungicidal *in vitro* activity of several LTPs, as well as the enhanced tolerance to bacterial pathogens by transgenic LTP expression and the induction of some isoforms by pathogens infection, have been illustrated [34, 36]. The recent identification of a putative LTP involved in systemic resistance signalling in *Arabidopsis* [37] and the binding of an LTP to a receptor implicated in the control of plant defence responses [38], support the role of this protein family in plant protection. LTPs are now classified in the pathogenesis-related (PR) protein families as PR-14 [39]. The involvement of LTPs in plant defence mechanisms is compatible with other possible functions postulated for these proteins. Particularly, a role in the assembly of extracellular lipophilic surface polymers, such as cutin and suberin, transporting their hydrophobic monomers to the outer layers of many aerial organs, has been reported [31]. A putative implication of LTPs in plant adaptation to abiotic stress (cold, salt) has also been described [29, 31].

Resistance to heat treatment and proteolytic digestion

The stability to digestion by the gastrointestinal tract, especially gastric pepsin, has been claimed as a characteristic of a true food allergen [24, 40]. This resistance to proteolysis would allow the allergens to maintain their immunogenic and allergenic motifs and thus to interact with the immune system associated with the gastrointestinal epithelia, thereby inducing both sensitization and systemic symptoms [24]. More-

over, stability to heat treatments (together with proteolytic resistance in some cases, such as in brewing) implies the presence of active allergen forms in processed foodstuffs [22, 41, 42] and beverages [43, 44]. LTPs show stability to both proteolytic and heat treatment.

The members of the family tested so far remain active after treatment with temperatures of up to 100 °C [23, 41, 43, 45]. Thus, *in vitro* IgE binding is maintained in peach Pru p 3 and maize Zea m 14 after 30 min at 121 °C [41] and after 160 min at 100 °C [23], respectively. Furthermore, *in vivo* positive responses (both in skin prick tests (SPTs) and oral challenges) have been obtained with boiled (100 °C for 30 min), and even overcooked (180 °C for 20 min) apple peel extracts containing Mal d 3 [45]. Besides, resistance to simulated gastric fluid and to acidic proteases of fungal origin has been shown for Pru p 3 [22, 41] and allergenic LTPs from barley [43] and carrot [9]. Proteolysis of Pru p 3 by pepsin, selected as a model system, is only detectable after 2 h of treatment, and even after 24 h around 40% of the allergen has not been degraded [22].

Their special stability mostly explains why LTPs have been identified as relevant allergens in beer (although based on a limited number of patients) [44, 46] and wine [47, 48], and why clinically significant levels of Pru p 3 have been found in peach-derived products (juice, nectar, jam) [22, 41]. Moreover, Zea m 14 causes allergic symptoms after eating heat-treated maize foodstuffs (polenta) [18, 23].

Plant food and pollen allergens belonging to the LTP family

According to several reports since 1992 [11, 49, 50], IgE binding electrophoretic bands in the range of 8–13 kDa have been detected in *Rosaceae* fruit extracts, when studying Mediterranean patients allergic to these fruits, with or without associated pollinosis. These bands were not inhibited by grass or birch pollen extracts in immunoblot inhibition assays [50], and seemed to be mainly confined to the fruit skin [49]. The first extensive articles on the identification of such bands as LTPs were published in 1999 by Spanish and Italian groups [14, 15]. Allergens of 9078 and 9138 Da were isolated from peach (*Prunus persica*) and apple (*Malus domestica*), and designated as Pru p 3 and Mal d 3, respectively [14]. Subsequent work on Pru p 3 has allowed an extensive characterization of this allergen, which is probably the best known allergenic LTP from plant foods to date. Thus, its full amino acid sequence has been determined at the protein level [51], its corresponding cDNA isolated [52], and its recombinant form, showing an immunologic activity equivalent to that of the natural allergen, expressed in the yeast *Pichia pastoris* [52, 53]. Furthermore, Pru p 3 sequential IgE epitopes have been mapped and a potential hypoallergenic triple point mutant obtained [54]. In addition, Pru p 3 levels in different peach-derived products have been quantified [22], and the *in vitro* and *in vivo* reactivities of Pru p 3 have been widely tested [12, 25, 55–58].

During the last 5 years, an increasing number of allergens belonging to the LTP family have been identified in several plant foods. The current information is particularly extensive in *Rosaceae* fruits, as expected. Besides Pru p 3 [14, 15] and Mal d 3 [14, 59], closely related allergens (>80% of sequence

identity) have been found in apricot (*Prunus armeniaca*; Pru ar 3) [16, 60], cherry (*Prunus avium*; Pru av 3) [17, 61] and plum (*Prunus domestica*; Pru d 3) [62] (see Fig. 2). Moreover, allergenic LTPs have also been identified in other fruits, such as grape (*Vitis vinifera*; Vit v 1) [48], in nuts, such as chestnut (*Castanea sativa*; Cas s 8) [25] and hazelnut (*Corylus avellana*; Cor a 8) [21, 42], in products derived from cereal seeds, such as maize (*Zea mays*; Zea m 17) [18] and barley (*Hordeum vulgare*; LTP1) [44], and in vegetables, such as asparagus (*Asparagus officinalis*; Aspa o 1.01 and Aspa o 1.02) [19], lettuce (*Lactuca sativa*; Lac s 1) [20], carrot (*Daucus carota*; Dau c LTP) [9] and probably onion (*Allium cepa*) [63] (see Fig. 2). Cross-reactions among allergens (LTPs) from *Rosaceae* fruits, and between these and allergens from other foods have been demonstrated both *in vitro* [9, 25, 50, 57] and *in vivo* [55]. In this context, immunoblot- and/or ELISA-inhibition assays suggest that allergens such as Cas s 8 or Zea m 14 share some common IgE epitopes with Pru p 3, but lack other main ones which are present in the peach allergen [18, 25].

In addition to foods, allergens belonging to the LTP family have also been identified in other plant sources. Thus, Hev b 12 from the latex of *Hevea brasiliensis* is a basic protein of 9.3 kDa, showing around 65% of sequence identity with the allergens from *Rosaceae* fruits mentioned above [64]. Preliminary *in vitro* data indicate cross-reactivity between Hev b 12 and the allergenic LTPs from peach and apple [64]. Moreover, some pollen allergens are also LTPs. Three of them, namely Art v 3 from *Artemisia vulgaris* [25], Ole e 7 from *Olea europaea* [26, 65], and a recently described *Arabidopsis thaliana* LTP [32], are proteins of 9–10 kDa, displaying 30–55% of sequence identity with allergenic food LTPs. In contrast, the major allergens of *Parietaria judaica*, Par j 1 and Par j 2, which show 45% of sequence identity with each other, present larger molecular sizes of 14.7 kDa (139 amino acids) and 11.3 kDa (102 amino acids), respectively [27, 28, 66]. However, both allergens conserve the 8 cysteine residues forming the 4 disulphide bridges typical of the LTPs (see Fig. 2), seem to fold in the characteristic all- α -type

structure [67, 68], and show moderate levels of identity with food LTPs in the common region of sequence (i.e. 28% between Par j 1 and Pru p 3). Cross-reactivity among the pollen and food allergens has been studied mainly in the case of Art v 3, which partially cross-reacts with Pru p 3 and Mal d 3, as shown by *in vitro* (ELISA-inhibition) and *in vivo* (SPT) assays [25, 55, 69].

Symptoms and populational profiles of sensitization to LTP allergens

The resistance of LTPs to gastric digestion and the sensitization of *Rosaceae* fruit-allergic patients without associated pollinosis to these allergens suggest that LTP sensitization occurs by the oral route [11, 24, 70]. This characteristic seems to be linked with a higher rate of severe and systemic symptoms than those induced by other plant food allergens, such as Bet v 1 homologues and profilin [24, 70]. Thus, a relationship between IgE to LTPs and systemic symptoms has been observed in patients allergic to cherry [61], peach [12], hazelnut [21, 42] and maize [18]. However, future studies including a larger population are needed to support this relationship on a solid statistical basis.

LTP sensitization shows a surprisingly, and yet unexplained, geographical distribution pattern. The allergen family is particularly relevant in the Mediterranean area (data only available for Spain and Italy to date), whereas its incidence in Central and Northern Europe seems to be very limited [70]. Positive SPT responses to peach Pru p 3 have been found in 60–90% of the Spanish patients with peach allergy [12, 55]. Furthermore, several surveys comprising Swiss, German, Danish, Spanish and/or Italian subjects have highlighted these differences. Thus, only 3% (3/101) of German patients, but 100% (7/7) of Italian patients, were sensitized to cherry Pru av 3, according to *in vitro* tests [17]. SPTs using r Pru av 3 in two groups of patients with cherry allergy confirmed by double-blind placebo-controlled food challenge support these results, revealing 4% (1/24) of positive responses

Foods	10	20	30	40	50	60	70	80	90	%id.			
Pru p 3	-ITCGQ-	VSSALAPCI	PIYVRGGCAVPPA-	CCNCIRNVN	NLARTTPDRQAACNCLK	QLSASVPGVNPNNAAALPGK-	CGVSI	PYKISASTNCA	TVK	--			
Mal d 3	-ITCGQ-	VSSALAPCI	GYVRSGCAVPPA-	CCNGIRTIN	GLARTTADROTACNCLNLAGS	ISGVNPNNAAALPGK-	CGVNV	PYKISTSTNCA	TVK	80			
Pru ar 3	-ITCGQ-	VSSSLAPCI	GYVRGGCAVPPA-	CCNCIRNVN	NLARTTPDRRTACNCLK	QLSGSISGVNPNNAAALPGK-	CGVNI	PYKISASTNCA	TVK	91			
Pru av 3	-LTCGQ-	VSSNLAPCI	AYVRGGCAVPPA-	CCNCIRNIN	NLAKTTADRTACNCLK	QLSASVPGVNPNNAAALPGK-	CGVNV	PYKISPTSTNCA	TVK	88			
Pru d 3	-ITCGQ-	VSSNLAPCI	PIYVRGGCAVPPA-	CCNCIRNVN	NLARTTPDRQAACNCLK	QLSASVPGVNPNNAAALPGK-	CGVNI	PYKISPTSTNCA	TVK	95			
Vit v 1	TVTCGQ-	VASALSPCI	SYLQKGCAVPPAG-	CCSGIKSL	NLSAAKTTPDRQAACKCLK	TFSSSVSGINYLASGLPGK-	CGVSV	PYKISPTSTDC	SKVT	61			
Cor a 8	SLTCGQ-	IKGNLTPO	VLYLKNGCVLPSP-	CCKGVRA	VNDASRTTSDRQSAACNCLK	QDTAKGIAGLNPNLAAGLPGK-	CGVNI	PYKISPTSTNC	NNVK	59			
Zea m 14	ATSCGQ-	VASATAPCI	SYARGQSGPSAG-	CCSGVRS	LNNAAARTTADERRACNCLN	AAAGVSGLNAGNAASIPSK-	CGVSI	PYKISTSTDC	SRVN	63			
Hor v LTP1	-LNCGQ-	VDSKMKP	CLTYVQGGPGPSGE-	CCNCVRD	LHNOAQSSGDROTVCNCLK	GIAIGHNLNLNNAASIPSK-	CNVNV	PYKISPTDIDC	SRIV	44			
Dau c LTP	-LTCGQ-	VTGALAPCI	GLYLRQVNVFVPLT	CCNVVR	GLNNAARTTLDKRTACGLK	QTANAVTGLNLNAAAGLPAR-	CGVNI	PYKISPTDIDC	NRIV	55			
Cas s 8	STTCGQ-	VSKSLMPCL	TYLKSNCGSPPGT	CCQCVKL*						46			
Asp o 1.01	-ITCGA-	DSKIG-	PGVSYVMGKCP	L*						43			
Asp o 1.02	-TSCGQ-	AVSMIS-	PCVNYARG*							50			
Lac s 1	ATSCGQ-	VTANLA*								64			
Latex													
Hev b 12	-ITCGQ-	VQSALVPCL	SYLKTTPPTPAT-	CCNCVRT	INNAAKTTPDRRTACQCLK	SAAGSVKGLNP	PTTVAGLPGK-	CGVNI	PYKISLSTNCA	TVK	65		
Pollens													
Par j 1	QETCGT-	MVRALMP	CLPFVQGKEKE	SKG-	CCSAKRLDGET	KGPQVHACEICQ	TAMKTYSDIDG	KLVSEV	PKH-	CGIVD	SKLPPIDV	NMDCKT	28
Par j 2	EEACCK-	VVDIMPL	CLHFKVGEKE	SKE-	CCSTKKLSEVVK	TEQKREACKCIV	RATKGISGINKEL	VAEV	PKK-	CDIKT	TLPPITAD	FDCKSI	27
Ara t LTP	ALSCGS-	VNSNLAA	CIYVLQGVIPPA-	CCSGVKN	LNSIAKTTPDRQAACNCI	QGAARALGSGLNAGRAAGIPKAC	GVNI	PYKISTSTNCK	TVR	53			
Art v 3	ALTCS-	DSNKS	PCLSYLQKGEV	EAD--	CCAGV	KGLND*							40
Ole e 7	APSQST-	VTALLTS	CVSYIDDQ*										19

Fig. 2. Alignment of amino acid sequences of food, latex and pollen allergens belonging to the lipid transfer protein (LTP) family. An asterisk indicates the end when only an N-terminal sequence is available. Percentages of sequence identity (% Id) with respect to Pru p 3 are shown on the right-hand side of the figure. The C-terminal domain of Par j 1 and Par j 2 are not included. See text for allergen designations.

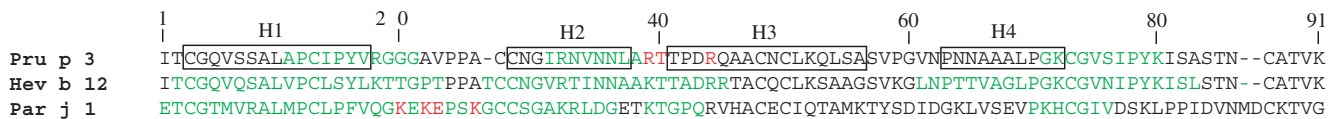


Fig. 3. Amino acid sequences of peach Pru p 3, latex Hev b 12 and *Parietaria* Par j 1 allergens, showing putative sequential IgE epitopes (in green) and critical residues for IgE binding (in red). The four predicted α -helices (H1–H4) in the three-dimensional (3-D) model of Pru p 3 (see Fig. 1) are indicated. The C-terminal domain of Par j 1 is not included.

in the Swiss group and 88% (8/9) in the Spanish one [61]. A similar pattern of sensitization has been suggested for hazelnut Cor a 8 [21, 42]. Interestingly, a recent report on *Parietaria judaica* pollen allergens [71] shows that Mediterranean patients sensitized to this pollen react mainly to r Par j 2 (a member of the LTP family), whereas non-Mediterranean patients are predominantly sensitized to other pollen allergens. The basis of the geographic differences in food LTP sensitization remains to be uncovered. Different eating habits and/or different genetic backgrounds between the Central/Northern and Southern European populations have been claimed to account for this.

Finally, it should be mentioned that a wide array of sensitization profiles to allergens of the LTP family has been described. Among peach-allergic patients, some are only sensitized to the *Rosaceae* fruit members Pru p 3 and Mal d 3, whereas others are reactive to these allergens in addition to LTPs from other foods (chestnut) and pollens (*Artemisia*) [55]. Moreover, a second route of sensitization, that is, by inhalation, is now arising, based on at least two different lines of evidence. Firstly, Pru p 3 inhalation from peach leaves could be prior to the clinical expression induced by the allergen via the oral route [72]. Secondly, a high prevalence (70%; 17/24) of Art v 3 has been detected in a group of patients selected by the criteria of primary allergy to *Artemisia* pollen, several of which also showed sensitization to peach (M. Lombardero et al., unpublished). Interestingly, Par j 1 does not seem to play a role in this cross-sensitization.

cDNA cloning and epitope mapping of LTP allergens

cDNA clones encoding several LTP allergens have been obtained, allowing not only to deduce the full amino acid sequence of the protein, but also to express it in heterologous systems, either prokaryotic (*Escherichia coli*) [17, 21, 27, 64] or eukaryotic (*P. pastoris*) [52]. The recombinant allergens produced in these systems can be useful tools for the diagnosis of allergy to food and/or pollen LTPs. However, a direct comparison of the recombinant and natural forms has not been carried out in most cases. Such a comparison was performed for peach Pru p 3, showing the equivalent folding, IgE binding, basophil activation, induction of sulphidoleukotrienes and histamine release capacity of the natural and recombinant version of the allergen [53].

In contrast with the increasing production of recombinant allergens, studies on the IgE epitopes of allergens belonging to the LTP family are very scarce, and no data on T cell epitopes of LTPs has been published to date. Pru p 3 is the only food member that has been investigated in this context. Its 3-D modelling has allowed to identify putative critical residues involved in IgE binding [54]. Three of them (positions R39, T40 and R44, located in the second loop

and the beginning of the third α -helix; see Fig. 3) were simultaneously mutated to alanine, generating the triple Pru p 3 mutant R39A/T40A/R44A. This mutant presents a drastic decrease (approximately 5-fold) in IgE binding capacity with respect to recombinant Pru p 3, thus suggesting that the three residues are included in a relevant IgE epitope [54]. Therefore, the triple mutant could be a starting point to produce hypoallergenic forms of Pru p 3 for future immunotherapy. On the other hand, the analysis of synthetic peptides covering the full Pru p 3 sequence, using sera from patients with peach allergy, has allowed to map sequential IgE epitopes in the regions 11–25, 31–45 and 71–80 of the Pru p 3 sequence (see Fig. 3 and ref. [54]). Interestingly, similar regions (residues 2–23, 27–46 and 62–84; Fig. 3) have been located in the latex LTP allergen Hev b 12 as potential IgE epitopes [73].

Among the allergenic pollen LTPs, the major *Parietaria judaica* allergen, Par j 1, has been cloned, expressed in *E. coli*, and analysed at the IgE epitope level [see ref. 66 for review]. The N-terminal domain of this allergen (residues 1–30) represents an immunodominant IgE epitope, which includes several essential residues for IgE binding, such as C14 and C29 (forming a disulphide bridge), and positions K21, K23, E24 and K27 [67]. Other parts of the Par j 1 sequence (residues 18–37, 41–47, 72–79 and 118–125) have also been identified as potential IgE epitopes [68]. Moreover, hypoallergenic variants of Par j 1 have been produced by disruption of its disulphide bridges (Cys/Ser substitutions) [74]. Although the sequential positions for putative IgE epitopes of Par j 1 and Pru p 3 show some similarities, both allergens present strong sequence divergence in most of these regions (see Fig. 3). Such divergence could explain the undetectable cross-reactivity between Pru p 3 and Par j 1 recently found in Spanish pollinic patients (M. Lombardero et al., unpublished).

Acknowledgements

We thank Dr L.F. Pacios for the 3-D model of Pru p 3 and D. Lamóneda for manuscript processing. Financial support was provided by Dirección General de Investigación, MCYT (project BMC2002–00196).

References

- Ma S, Sicherer SH, Nowak-Węgrzyn A. A survey on the management of pollen-food allergy syndrome in allergy practices. *J Allergy Clin Immunol* 2003; 112:784–8.
- Eriksson NE, Formgren H, Svenonius E. Food hypersensitivity in patients with pollen allergy. *Allergy* 1982; 37:437–43.
- Ebner C, Hirschwehr R, Bauer L et al. Identification of allergens in fruits and vegetables. IgE cross reactivities with important birch

- pollen allergens Bet v 1 and Bet v 2 (birch profilin). *J Allergy Clin Immunol* 1995; 95:962–9.
- 4 Ortolani C, Ballmer-Weber BK, Skamstrup Hansen K et al. Hazelnut allergy: a double-blind, placebo-controlled food challenge multicenter study. *J Allergy Clin Immunol* 2000; 105:577–81.
 - 5 Vieths S, Scheurer S, Ballmer-Weber B. Current understanding of cross-reactivity of food allergens and pollen. *Ann NY Acad Sci* 2002; 964:47–68.
 - 6 Van Ree R. Carbohydrate epitopes and their relevance for the diagnosis and treatment of allergic diseases. *Int Arch Allergy Immunol* 2002; 129:189–97.
 - 7 Kleine-Tebbe J, Vogel L, Crowell DN, Hanstein UF, Vieths S. Severe oral allergy syndrome and anaphylactic reactions caused by a Bet v 1-related PR-10 protein in soybean, SAM22. *J Allergy Clin Immunol* 2002; 110:797–804.
 - 8 Mittag D, Vieths S, Vogel L et al. Soybean allergy in patients allergic to birch pollen: clinical investigation and molecular characterization of allergens. *J Allergy Clin Immunol* 2004; 113:148–54.
 - 9 Asero R, Mistrello G, Roncarolo D et al. Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. *Int Arch Allergy Immunol* 2000; 122:20–32.
 - 10 Rodriguez-Perez R, Crespo JF, Rodríguez J et al. Profilin is a relevant melon allergen susceptible to pepsin digestion in patients with oral allergy syndrome. *J Allergy Clin Immunol* 2003; 111:634–9.
 - 11 Fernandez-Rivas M, van Ree R, Cuevas M. Allergy to *Rosaceae* fruits without related pollinosis. *J Allergy Clin Immunol* 1997; 100:728–33.
 - 12 Fernández-Rivas M, González-Mancebo E, Rodríguez-Pérez R et al. Clinically relevant peach allergy is related to peach lipid transfer protein, Pru p 3, in the Spanish population. *J Allergy Clin Immunol* 2003; 112:789–95.
 - 13 Cuesta-Herranz J, Lazaro M, de las Heras M et al. Peach allergy pattern: experience in 70 patients. *Allergy* 1998; 53:78–82.
 - 14 Sánchez-Monge R, Lombardero M, García-Sellés FJ et al. Lipid-transfer proteins are relevant allergens in fruit allergy. *J Allergy Clin Immunol* 1999; 103:514–9.
 - 15 Pastorello EA, Farioli L, Pravettoni V et al. The major allergen of peach (*Prunus persica*) is a lipid transfer protein. *J Allergy Clin Immunol* 1999; 103:520–6.
 - 16 Pastorello EA, D'Ambrosio FP, Pravettoni V et al. Evidence for a lipid transfer protein as the major allergen of apricot. *J Allergy Clin Immunol* 2000; 105:371–7.
 - 17 Scheurer S, Pastorello EA, Wangorsch A, Kästner M, Hausteine D, Vieths S. Recombinant allergens Pru av 1 and Pru av 4 and a newly identified lipid transfer protein in the *in vitro* diagnosis of cherry allergy. *J Allergy Clin Immunol* 2001; 107:724–31.
 - 18 Pastorello EA, Farioli L, Pravettoni V et al. The maize major allergen, which is responsible for food-induced allergic reactions, is a lipid transfer protein. *J Allergy Clin Immunol* 2000; 106:744–51.
 - 19 Díaz-Perales A, Tabar AI, Sánchez-Monge R et al. Characterization of asparagus allergens: a relevant role of lipid transfer proteins. *J Allergy Clin Immunol* 2002; 110:790–6.
 - 20 San Miguel-Moncin M, Krail M, Scheurer S et al. Lettuce anaphylaxis: identification of a lipid transfer protein as the major allergen. *Allergy* 2003; 58:511–7.
 - 21 Schocker F, Lüttkopf D, Scheurer S et al. Recombinant lipid transfer protein Cor a 8 from hazelnut: a new tool for *in vitro* diagnosis of potentially severe hazelnut allergy. *J Allergy Clin Immunol* 2004; 113:141–7.
 - 22 Duffort OA, Polo F, Lombardero M et al. Immunoassay to quantify the major peach allergen Pru p 3 in foodstuffs. Differential allergen release and stability under physiological conditions. *J Agric Food Chem* 2002; 50:7738–41.
 - 23 Pastorello EA, Pompei C, Pravettoni V et al. Lipid-transfer protein is the major maize allergen maintaining IgE-binding activity after cooking at 100 °C, as demonstrated in anaphylactic patients with positive double-blind, placebo-controlled food challenge results. *J Allergy Clin Immunol* 2003; 112:775–83.
 - 24 Van Ree R. Clinical importance of non-specific lipid transfer proteins as food allergens. *Biochem Soc Trans* 2002; 30:910–3.
 - 25 Díaz-Perales A, Lombardero M, Sánchez-Monge R et al. Lipid-transfer proteins as potential plant panallergens: cross-reactivity among proteins of *Artemisia* pollen, *Castanea* nut and *Rosaceae* fruits, with different IgE-binding capacities. *Clin Exp Allergy* 2000; 30:1403–10.
 - 26 Tejera ML, Villalba M, Batanero E, Rodríguez R. Identification, isolation, and characterization of Ole e 7, a new allergen of olive tree pollen. *J Allergy Clin Immunol* 1999; 104:797–802.
 - 27 Costa MA, Colombo P, Izzo V et al. cDNA cloning, expression and primary structure of Par j I, a major allergen of *Parietaria judaica* pollen. *FEBS Lett* 1994; 341:182–6.
 - 28 Duro G, Colombo P, Costa MA et al. cDNA cloning, sequence analysis and allergological characterization of Par j 2.0101, a new major allergen of the *Parietaria judaica* pollen. *FEBS Lett* 1996; 399:295–8.
 - 29 Kader JC. Lipid-transfer proteins in plants. *Annu Rev Plant Physiol Plant Mol Biol* 1996; 47:627–54.
 - 30 Kader JC. Lipid-transfer proteins: a puzzling family of plant proteins. *Trends Plant Sci* 1997; 2:66–70.
 - 31 Douliet JP, Michon T, Elmorjani K, Marion D. Structure, biological and technological functions of lipid transfer proteins and indolines, the major lipid binding proteins from cereal kernels. *J Cereal Sci* 2000; 32:1–20.
 - 32 Chardin H, Mayer C, Senechal H et al. Lipid transfer protein 1 is a possible allergen in *Arabidopsis thaliana*. *Int Arch Allergy Immunol* 2003; 131:85–90.
 - 33 Botton A, Begheldo M, Rasori A, Bonghi C, Tonutti P. Differential expression of two lipid transfer protein genes in reproductive organs of peach (*Prunus persica* L. Batsch). *Plant Sci* 2002; 163:993–1000.
 - 34 García-Olmedo F, Molina A, Segura A, Moreno M. The defensive role of nonspecific lipid-transfer proteins in plants. *Trends Microbiol* 1995; 3:72–4.
 - 35 Fernández-Rivas M, Cuevas M. Peels of *Rosaceae* fruits have a higher allergenicity than pulps. *Clin Exp Allergy* 1999; 29:1239–47.
 - 36 Molina A, García-Olmedo F. Enhanced tolerance to bacterial pathogens caused by the transgenic expression of barley lipid transfer protein LTP2. *Plant J* 1997; 12:669–75.
 - 37 Maldonado AM, Doerner P, Dixon RA, Lamb CJ, Cameron RK. A putative lipid transfer protein involved in systemic resistance signalling in *Arabidopsis*. *Nature* 2002; 419:399–403.
 - 38 Buhot N, Douliet JP, Jacquemard A et al. A lipid transfer protein binds to a receptor involved in the control of plant defence responses. *FEBS Lett* 2001; 509:27–30.
 - 39 Van Loon LC, van Strien EA. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiol Mol Plant Pathol* 1999; 55:85–97.
 - 40 Astwood JD, Leach JN, Fuchs RL. Stability to food allergens to digestion *in vitro*. *Nature Biotechnol* 1996; 14:1269–73.
 - 41 Brenna O, Pompei C, Ortolani C, Pravettoni V, Farioli L, Pastorello EA. Technological processes to decrease the allergenicity of peach juice and nectar. *J Agric Food Chem* 2000; 48:493–7.
 - 42 Pastorello EA, Vieths S, Pravettoni V et al. Identification of hazelnut major allergens in sensitive patients with positive double-blind, placebo-controlled food challenge results. *J Allergy Clin Immunol* 2002; 109:563–70.
 - 43 Lindorff-Larsen K, Winther JR. Surprisingly high stability of barley lipid transfer protein, LTP1, towards denaturant, heat and proteases. *FEBS Lett* 2001; 488:145–8.
 - 44 García-Casado G, Crepo JF, Rodríguez J, Salcedo G. Isolation and characterization of barley lipid transfer protein and protein Z as beer allergens. *J Allergy Clin Immunol* 2001; 108:647–9.

- 45 Asero R, Mistrello G, Roncarolo D, Amato S, Falagiani P. Analysis of the heat stability of lipid transfer protein from apple. *J Allergy Clin Immunol* 2003; 112:1009–11.
- 46 Asero R, Mistrello G, Roncarolo D, Amato S, van Ree R. A case of allergy to beer showing cross-reactivity between lipid transfer proteins. *Ann Allergy Asthma Immunol* 2001; 87:65–7.
- 47 García-Robaina JC, de la Torre-Morin F, Sánchez-Machín I, Sánchez-Monge R, Barber D, Lombardero M. Anaphylaxis induced by exercise and wine. *Allergy* 2001; 56:357–8.
- 48 Pastorello EA, Farioli L, Pravettoni V et al. Identification of grape and wine allergens as an endochitinase 4, a lipid-transfer protein, and a thaumatin. *J Allergy Clin Immunol* 2003; 111:350–9.
- 49 Leonart R, Cistero A, Carreira J, Batista A, Moscoso del Prado J. Food allergy: identification of the major IgE-binding component of peach (*Prunus persica*). *Ann Allergy* 1992; 69:128–30.
- 50 Pastorello EA, Ortolani C, Farioli L 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.
- 51 Pastorello EA, Ortolani C, Baroglio C et al. Complete amino acid sequence determination of the major allergen of peach (*Prunus persica*) Pru p 1. *Biol Chem* 1999; 380:1315–20.
- 52 Díaz-Perales A, García-Casado G, Sánchez-Monge R, García-Selles FJ, Barber D, Salcedo G. cDNA cloning and heterologous expression of the major allergens from peach and apple belonging to the lipid-transfer protein family. *Clin Exp Allergy* 2002; 32:87–92.
- 53 Díaz-Perales A, Sanz ML, García-Casado G et al. Recombinant Pru p 3 and natural Pru p 3, a major peach allergen, show equivalent immunologic activity: a new tool for the diagnosis of fruit allergy. *J Allergy Clin Immunol* 2003; 111:628–33.
- 54 García-Casado G, Pacios LF, Díaz-Perales A et al. Identification of IgE-binding epitopes of the major peach allergen Pru p 3. *J Allergy Clin Immunol* 2003; 112:599–605.
- 55 García-Sellés FJ, Díaz-Perales A, Sánchez-Monge R et al. Patterns of reactivity to lipid transfer proteins of plant foods and *Artemisia* pollen: an *in vivo* study. *Int Arch Allergy Immunol* 2002; 128:115–22.
- 56 Asero R, Mistrello G, Roncarolo D, Casarini M, Falagiani P. Allergy to non-specific lipid transfer proteins in *Rosaceae*: a comparative study of different *in vivo* diagnostic methods. *Ann Allergy Asthma Immunol* 2001; 87:68–71.
- 57 Asero R, Mistrello G, Roncarolo D et al. Immunological cross-reactivity between lipid transfer proteins from botanically unrelated plant-derived foods: a clinical study. *Allergy* 2002; 57:900–6.
- 58 Asero R, Mistrello G, Roncarolo D, Amato S. Relationship between peach lipid transfer protein specific IgE levels and hypersensitivity to non-Rosaceae vegetable foods in patients allergic to lipid transfer protein. *Ann Allergy Asthma Immunol* 2004; 92:268–72.
- 59 Pastorello EA, Pravettoni V, Farioli L et al. Clinical role of a lipid transfer protein that acts as a new apple-specific allergen. *J Allergy Clin Immunol* 1999; 104:1099–106.
- 60 Conti A, Fortunato D, Ortolani C et al. Determination of the primary structure of two lipid transfer proteins from apricot (*Prunus armeniaca*). *J Chromatogr B* 2001; 756:123–9.
- 61 Ballmer-Weber BK, Scheurer S, Fritsche P et al. Component-resolved diagnosis with recombinant allergens in patients with cherry allergy. *J Allergy Clin Immunol* 2002; 110:167–73.
- 62 Pastorello EA, Farioli L, Pravettoni V et al. Characterization of the major allergen of plum as a lipid transfer protein. *J Chromatogr B* 2001; 756:95–103.
- 63 Asero R, Mistrello G, Roncarolo D, Amato S. A case of onion allergy. *J Allergy Clin Immunol* 2001; 108:309–10.
- 64 Beezhold DH, Hickey VL, Kostyal DA et al. Lipid transfer protein from *Hevea brasiliensis* (Hev b 12), a cross-reactive latex protein. *Ann Allergy Asthma Immunol* 2003; 90:439–45.
- 65 Rodríguez R, Villalba M, Batanero E et al. Allergenic diversity of the olive pollen. *Allergy* 2002; 57:6–16.
- 66 Colombo P, Bonura A, Costa MA et al. The allergens of *Parietaria*. *Int Arch Allergy Immunol* 2003; 130:173–9.
- 67 Colombo P, Kennedy D, Ramsdale T et al. Identification of an immunodominant IgE epitope of the *Parietaria judaica* major allergen. *J Immunol* 1998; 160:2780–5.
- 68 Asturias JA, Gómez-Bayón N, Eseverri JL, Martínez A. Par j 1 and Par j 2, the major allergens from *Parietaria judaica* pollen, have similar immunoglobulin E epitopes. *Clin Exp Allergy* 2003; 33:518–24.
- 69 Pastorello EA, Pravettoni V, Farioli L et al. Hypersensitivity to mugwort (*Artemisia vulgaris*) in patients with peach allergy is due to a common lipid transfer protein allergen and is often without clinical expression. *J Allergy Clin Immunol* 2002; 110:310–7.
- 70 Ballmer-Weber BK. Lipid transfer protein as a potential panallergen? *Allergy* 2002; 57:873–5.
- 71 Stumvoll S, Westritschnig K, Lidholm J et al. Identification of cross-reactive and genuine *Parietaria judaica* pollen allergens. *J Allergy Clin Immunol* 2003; 111:974–9.
- 72 García BE, Lombardero M, Echechipia S et al. Respiratory allergy to peach leaves and lipid transfer proteins. *Clin Exp Allergy* 2004; 34:291–5.
- 73 Beezhold DH, Hickey VL, Zuidmeer L, Sussman GL. Epitope analysis of Hev b 12, a cross reacting latex allergen. *J Allergy Clin Immunol* 2003; 111 (Suppl. 2), Abstract 1032.
- 74 Bonura A, Amoroso S, Locorotondo G et al. Hypoallergenic variants of the *Parietaria judaica* major allergen Par j 1: a member of the non-specific lipid transfer protein plant family. *Int Arch Allergy Immunol* 2001; 126:32–40.