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Research paper

The lipid transfer proteins (LTP) essentially concentrate in the skin of Rosaceae fruits as cell surface exposed allergens

J.-P. Borges[a], A. Jauneau[a], C. Brulé[a], R. Culerrier[a], A. Barre[a], A. Didier[b], P. Rougé[a,*]

[a] *Surfaces cellulaires et signalisation chez les végétaux, UMR UPS-CNRS 5546, 24, chemin de Borde-Rouge, 31326 Castanet-Tolosan, France*
[b] *Clinique des voies respiratoires, Centre Hospitalier Universitaire Larrey, 24, chemin de Pourvourville, 31059 Toulouse cedex 09, France*

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Abstract

The localization and distribution of non-specific lipid transfer proteins (nsLTP) allergens in the skin and pulp of Rosaceae fruits (apple, peach, apricot, plum) has been investigated. nsLTP essentially concentrate in the pericarp of the fruits whereas the pulp contains lower amounts of allergens. Immunolocalization showed they are primarily located in the cytosol but are subsequently excreted and finally accumulate at the plasmalemma–cell wall interface and in the cell wall. However, high discrepancies were observed in the content of allergens among, e.g. different cultivars of apple. As a consequence, the consumption of peeled-off fruits is recommended to reduce the risk of severe allergic reactions (anaphylactic shock) in individuals sensitized to Rosaceae fruits.

Keywords: Mal d 3; Pru p 3; Pru d 3; Pru ar 3; Allergen; Lipid transfer protein; Rosaceae fruit

1. Introduction

Non-specific lipid transfer proteins (nsLTP) have been recently introduced as major allergens in food allergy, especially in allergies to the Rosaceae (apple, apricot, peach, plum) fruits [1–8]. Plant nsLTP consist of small-sized basic proteins (p$I \geq 9$) of either 91 (LTP2 group) or fewer (LTP1 group) amino acid residues that belong to family 14 of pathogenesis-related (PR-14) proteins [9]. nsLTP allergens, which essentially occur in LTP2 group, consist of a core made of four α-helices stabilized by four conserved disulfide bridges and a loosely coiled C-terminal tail [10–18]. This tightly packed three-dimensional structure accounts for the extreme resistance of nsLTP to both thermal denaturation and proteolysis [19–22]. Accordingly, nsLTP from food products trigger hypersensitive reactions, e.g. contact dermatitis, in individuals previously sensitized by pollen nsLTP but are also capable to achieve the sensitization of susceptible individuals via a stimulation of the lymph nodes of the gastro-intestinal tract immune system [23,24]. The eventual occurrence of allergens at the cell surface is of particular importance to promote contact dermatitis reactions in individuals sensitized to fruit allergens [25]. Previous measurements have pointed out some discrepancies among the amount of nsLTP in the skin and pulp of apple fruit depending on the apple varieties [26]. In most of the varieties, the skin of apple fruits exhibited the higher content in nsLTP. In the present work, we completed these previous informations by focusing our attention on the content of LTP in different Rosaceae fruits (apple, peach, apricot, plum) and, especially, on the localization of nsLTP at the cell surface. With the exception of the plum fruits, the skin of other Rosaceae fruits usually contains the higher amounts of nsLTP and, more interestingly, most of the allergen occurs at the cell surface and is therefore directly available to trigger contact dermatitis in sensitized people. Such a particular localization accounts for the unexpected quick allergenic response of sub-

Abbreviations: Ab, antibodies; ELISA, enzyme linked immuno-sorbent assay; LTP, lipid transfer protein; nsLTP, non-specific lipid transfer protein; Mal d 3, *Malus domestica* (apple) allergen 3; PBSTM, 10 mM phosphate buffered saline (pH 7.4) containing 0.2% (v/v) Tween 20 and 5% (v/v) defated milk; Pru ar 3, *Prunus armeniaca* (apricot) allergen 1; Pru d 3, *Prunus domestica* (plum) allergen 1; Pru p 3, *Prunus persica* (peach) allergen 3; PVP, polyvinyl pyrrolidone; TBS, 20 mM Tris buffered saline (pH 7.5).

* Corresponding author. Fax: +33 5 62 19 35 02.
E-mail address: rouge@scsv.ups-tlse.fr (P. Rougé).

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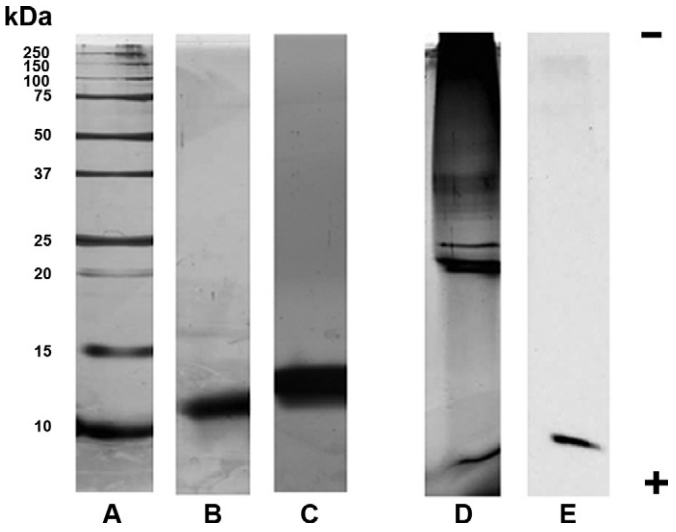

Fig. 1. Silver nitrate-stained SDS-PAGE analysis of purified Pru p 3 (B), Mal d 3 (C), soluble proteins of peach (D), and molecular weight markers (A). Immunoblot analysis of total soluble proteins from peach (E) showing that the rabbit anti-Pru p 3 polyclonal antibodies recognize a single band and does not react with any other protein fraction.

jects previously sensitized to peach upon a simple skin contact with the fruit.

2. Results

As shown from immuno-tissue-prints (ITP) experiments, nsLTP are essentially concentrated in the skin of apple and peach fruits whereas this allergen seems to be similarly distributed in both the skin and pulp of apricot and plum fruits (Fig. 2). Enzyme linked immuno-sorbent assay (ELISA) measurements confirm that the skin of peach and apple contains the higher amounts of nsLTP but discrepancies occur between different fruit varieties (Table 1). Especially in peach, the downy skin of the fruit consists of short hairs extremely rich in LTP as shown from immuno-cytochemical observations (Figs. 3H, K and 4D). In fact, the LTP amount of the skin is underestimated since the fruits were carefully washed before preparing the protein extracts that should eliminate most of the LTP-rich downy cover of the fruits. Accordingly, the LTP content of the skin measured on unwashed peach fruits was definitely higher (~8.4 μg g^{-1}) than that measured for washed skin (~5.2 μg g^{-1}). The ratio of the nsLTP content in the skin and pulp thus reaches 2.5 instead of 1.6. Very similar results were reported for other peach varieties [25]. The discrepancies observed between different varieties of apple are extremely important. Compared to a few varieties (Chanteclerc, Golden delicious, Granny Smith) where the content of nsLTP of the skin amounts from 10- up to 30-times that measured in the pulp, the ratio of the nsLTP content in the skin and pulp of other apple varieties varies from 2 to 4 (Table 1).

The immuno-cytochemical localization of nsLTP in the different parts (epidermis, hypodermis, parenchyma) of the fruit pericarp has been documented using polyclonal rabbit antibodies (Ab) specifically directed against Pru p 3 and Mal d 3

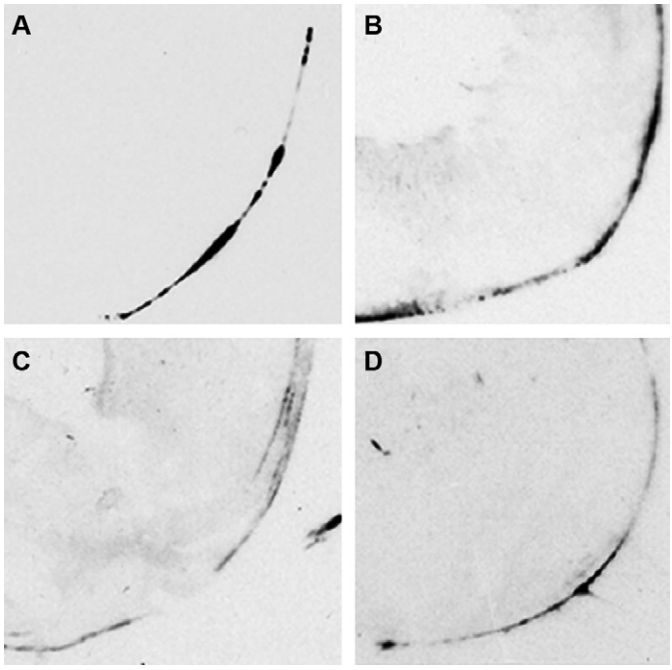

Fig. 2. ITP performed on apple (Golden delicious) (A), peach (B), yellow plum (C) and red plum (D).

Table 1
LTP content (expressed as μg g^{-1} tissue) in the skin and pulp of fruits

Fruit	LTP content of skin (μg g^{-1} tissue)	LTP content of pulp (μg g^{-1} tissue)	Ratio skin/pulp
Royal Gala	3.3	1.2	2.8
Bertanne	2	0.6	3.3
Pink Lady	7.9	1.1	7.1
Granny Smith	17.3	0.5	34.6
Chanteclerc	12.4	1.2	10.3
Golden delicious	12.0	0.9	13.3
Fuji	9	1.1	8.2
Red Chief	3.4	1.8	1.9
Reinette Canada	2	0.5	4
Apricot	1.8	1.6	1.1
Peach*	8.4	3.4	2.5
Peach**	5.2	3.2	1.6
Yellow plum	1.5	1.7	0.9
Red plum	1.5	1.7	0.9

Measurements performed on unwashed (*) and washed (**) peach skin.

(Fig. 1). Confocal laser scanning microscopy observations of apple samples showed that nsLTP labeling essentially occurred in the cytoplasm in contact with the cell wall (Fig. 3G, J). In addition, the labeling strongly decreased from the pericarp and, especially, from the hypodermal cells to the parenchyma cells of the mesocarp. A rather heterogeneous and irregular labeling was observed in peach and plum samples, with spots of fluorescence distributed within the cytoplasm and the inner part of the cell wall (Fig. 3H–L). Especially in peach, a very strong labeling was associated to the thick wall of the downy hairs covering the epidermal cell layer (Fig. 3H, K).

A more detailed subcellular localization of nsLTP in fruit was carried out by electron miscroscopy using immuno-gold labeling. Although some discrepancies occurred among the dif-