

Nonspecific lipid-transfer proteins in plant foods and pollens: an important allergen class

Heimo Breiteneder^a and Clare Mills^b

Purpose of review

Here we focus our attention on the structural stability and physicochemical properties of plant nonspecific lipid-transfer proteins (nsLTPs) as keys to their allergenicity. We further present the current opinions on the route of sensitization and include the latest additions to the nsLTP allergen family.

Recent findings

Plant nsLTPs are small cysteine-rich lipid-binding proteins that play a key role in plant resistance to biotic and abiotic stress. Besides their relevance for plant–pathogen interactions, nsLTPs have attracted interest as true food allergens which are of high importance to atopsics in Mediterranean countries. It is now becoming clear that their molecular properties such as the remarkable stability to proteolysis and thermal denaturation are intrinsically linked to their allergenicity. These properties also facilitate sensitization via the gastrointestinal tract which allows these molecules to act as allergens independently of prior exposure to pollen. In addition, a group of allergenic pollen nsLTPs exists which seem to be only partially linked to the food nsLTPs by cross-reactivity.

Summary

Research into the family of nsLTPs will continue to provide insights about the particular molecular properties that make an nsLTP an allergen and how primary sensitization occurs.

Keywords

plant-food and pollen nonspecific lipid-transfer protein, plant-food and pollen nsLTP, plant–pathogen interaction, route of sensitisation, stability, true food allergen

Introduction

An increasing number of proteins have been characterised as allergens in recent years. Many of the plant-food and pollen allergens fall into only a few protein families [1•,2•]. Plant nonspecific lipid-transfer proteins (nsLTPs), by virtue of the presence of an eight-cysteine motif, belong to a diverse group of around 500 proteins, not all of which by far are allergens [3••]. Interestingly, nsLTPs are members of the prolamin superfamily which includes a range of important plant allergens [4]. nsLTPs as defined by their function are also members of the heterogeneous collection of pathogenesis-related proteins [5]. In contrast to pollen-related food allergy, sensitization to plant-food nsLTPs occurs independently from birch pollinosis and frequently results in systemic and severe reactions [6]. Almost all study populations where nsLTPs were described as relevant allergens were from Mediterranean countries [7••,8]. In this context, much effort has been focused on the study of nsLTPs as model allergens of true food allergy, and less so on the allergenic pollen nsLTPs. The recent findings on the biochemical and allergenic characteristics with a focus on structure, function and stability are reviewed here.

The structure and function of nonspecific lipid-transfer proteins

Plant nsLTPs are classified into two subfamilies according to their molecular masses – nsLTP1 ($M_r \sim 9000$) and nsLTP2 ($M_r \sim 7000$) – which are unique to flowering plants [9,10]. nsLTPs have been purified from many plant species, with well over 100 having been sequenced on the nucleic acid level to date [3••]. Comparisons of these sequences show that the nsLTPs are closely homologous basic proteins. All nsLTPs possess eight conserved cysteines and are stabilized by four intrachain disulphide bonds. This pattern of disulphide bonds is a feature of the prolamin superfamily, with characteristic Cys–Cys and Cys–Xaa–Cys motifs, where Xaa represents any other residue, which are shared with other members of the superfamily including the seed-storage prolamins of cereals, the 2 S albumins and members of the α -amylase/trypsin inhibitors of cereals [1•]. nsLTPs are a dominant plant protein family but since only members of the nsLTP1 family have been characterized as allergens, these will be the focus of this review.

nsLTPs have been well defined at a structural level with the first structure being defined for maize [11]

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^aDepartment of Pathophysiology, Medical University of Vienna, Vienna, Austria and

^bInstitute of Food Research, Norwich, UK

Correspondence to Heimo Breiteneder, PhD, Department of Pathophysiology, Medical University of Vienna, AKH-EBO-3Q, Waehringer Guertel 18-20, 1090 Vienna, Austria

Tel: +431 40400 5102; Fax: +431 40400 5130;

e-mail: Heimo.Breiteneder@meduniwien.ac.at

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Abbreviation

nsLTP nonspecific lipid-transfer protein

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and subsequently for homologues from a number of cereals including rice [12], barley [13] and wheat, the latter having been studied binding to a variety of hydrophobic ligands including prostaglandins [14]. All are characterized by a related fold consisting of bundles of four α -helices stabilized by disulphide bonds with a central hydrophobic tunnel into which lipophilic molecules can bind [15^{*}]. The disulphide-bonding pattern is essential for the existence of this tunnel [16], which shows considerable plasticity, underlying the ability of nsLTPs to accommodate a diverse range of ligands in the tunnel ranging from sphingolipids, to prostaglandins, to amphotericin B and other hydrophobic drugs [14,17]. The free and lipid-bound forms of nsLTPs show structural differences, most notably in the C-terminal residues 75–84 of the wheat nsLTP, although smaller changes are observed in the same region of the maize nsLTP on binding lipid.

Lipid-transfer proteins have been suggested to mediate the transfer of phospholipids between vesicles and membranes [18]. Proteins with such in-vitro activities are widely found in plants, mammals, fungi and some bacteria and were speculated to play a role in managing the flux of lipids essential for the genesis and renewal of cellular membranes. They can be divided into two types: those which are specific for certain classes of phospholipids, and those (called nonspecific) that are able to accommodate several classes of lipids. It is now emerging that plants have used the three-dimensional scaffold of the nsLTPs in a promiscuous fashion and that many nsLTPs are not able to transfer lipids, and indeed in one instance the hydrophobic tunnel has been lost completely [19]. Today, all the structural, biochemical and physiological information has confirmed that nsLTPs are not involved in intracellular lipid trafficking [20]. Indeed lipid-transfer proteins may play an important role in plant defence against fungi and bacteria, the exact mechanism of action still being unknown [19,21–23]. Additional data also suggest a role in somatic embryogenesis [24] and in the formation of the surface layers cutin and suberin which protect plant organs against biotic and abiotic stress [25]. LTPs have been included among the pathogenesis-related proteins as family 14 [5].

The structural stability of nonspecific lipid-transfer proteins and its impact on sensitisation and elicitation of allergic reactions

Two properties that seem in general to be shared by plant protein allergens are abundance coupled with structural stability [26]. While not very abundant in the plant tissues as a whole, levels of nsLTPs are relatively abundant in skin and peel, being around 7-fold higher in these tissues compared with pulp in fruits such as peach [27]. It is becoming evident that nsLTPs share remarkable stability

to both thermal denaturation and proteolysis, despite lacking a classical well-packed hydrophobic core. Molecular dynamics simulations indicate that the disulphide skeleton is an important structural feature conferring this stability, disruption of the disulphide bonds resulting in a loss of the lipid-binding tunnel [16]. For example, the nsLTP of barley only begins to unfold following heating above 95°C [28] and generally the proteins refold on cooling.

Given their small size only limited proteolysis might be expected to be sufficient for nsLTPs to become reduced to immunologically insignificant fragments. However, the disulphide-bonded structure of members of the prolamin superfamily can hold such fragments together in a much larger molecule, as has been shown for the 2 S albumin allergen from Brazil nut *Ber e 1* [29]. In addition the compactness of nsLTPs makes them poor substrates for proteases such as pepsin and trypsin despite having a number of internal tryptic cleavage sites [28,30]. The inherent stability of this scaffold also undoubtedly underlies the ability of these allergens to retain their allergenic activity in processed foods such as sterilized peach juice [31], beer [32], baked or boiled apple [33], and fermented products such as wine [34]. Thermal processing does not alter their clinical reactivity, as indicated by the ability of well-cooked polenta to retain its ability to trigger reactions in individuals reactive to maize nsLTP following oral challenge [35]. Recently it was shown that the strong allergenicity of cherry nsLTPs was not affected by protease treatment or thermal processing of fruits [36^{**}].

Route of sensitisation

There is some debate as to whether allergies triggered by nsLTPs in plant foods are actually the result of prior sensitisation to pollen nsLTPs. Interestingly, the levels of similarity between food and pollen nsLTPs (with regards to both sequence and structure) are not as pronounced as in the *Bet v 1* pollen fruit allergen family. Symptoms in patients from central Spain allergic to apples, peaches and/or pears but without pollinosis were severe whereas oral symptoms were less frequent [37]. When the clinical reactivity to peach was established in another Spanish study population by double-blind, placebo-controlled food challenges, it was shown that Pru p 3 was the major allergen of peach and that the IgE response to this allergen was related to the clinical expression of peach allergy [38]. Sensitisation to profilin was observed in those patients with an associated pollen allergy but did not appear to be related to the clinical reactivity to peach [38]. In general, several studies have clearly demonstrated a higher incidence of systemic reactions in LTP-sensitised food allergic individuals than in patients with pollen-related food allergies. A group of Spanish patients with positive skin-prick tests to peach profilin

but not Pru p 3 showed a bias towards symptoms linked to oral allergy syndrome and fewer systemic reactions than the Pru p 3-positive group [39].

The status of nsLTPs as true food allergens is further supported by a growing body of studies. Rosaceae nsLTPs were demonstrated to cross-react with a broad range of vegetable foods and many of these cross-reactivities were accompanied by clinical food allergy, frequently including systemic reactions [30]. nsLTPs seem to be able to sensitise directly via the gastrointestinal tract. Certainly resistance to processing and to digestion would be important in allowing sufficient immunologically reactive fragments to be taken up and sensitise the gastrointestinal immune system in such instances. Maize nsLTP maintained its IgE-binding capacity after heat treatment, thus being the most plausible candidate for causing severe anaphylactic reactions to cooked maize [35]. nsLTPs from grape were shown to survive the brewing process and to be present and allergenic in wine [34]. The consensus now seems to be that sensitisation to food nsLTPs is not an epiphenomenon of an exposure to pollen but is caused by consumption of the respective food. Yet, there is evidence of IgE cross-reactivity between the peach nsLTP Pru p 3 and the mugwort pollen nsLTP Art v 3, and there is some debate on whether peach or pollen is the primary sensitising agent. Lombardero *et al.* [40] have evidence that in some patients with IgE to Art v 3 and Pru p 3, Art v 3 behaved as the primary sensitising agent. Even among the food nsLTPs there is a remarkable gradient of similarity from peach to maize to wheat when one regards the topography of the surface that is able to bind IgE [2•].

nsLTPs have been described as predominant Rosaceae fruit allergens in Mediterranean populations but not for central and northern European ones. There are two possible scenarios to explain these findings. On one hand nsLTPs are capable to sensitise by the ingestion route [30]. In this case, sensitisation to nsLTP of peach seems to be the consequence of its much higher consumption over a much longer period per year in the Mediterranean area. On the other hand, Art v 3, a major mugwort allergen and nsLTP, can act as the primary sensitising allergen in some patients [40]. Pollen grains of mugwort are an important cause of pollinosis in weed-rich areas of the Mediterranean basin, but not in northern Europe. Patients with high exposure to *Artemisia* pollen have a high frequency of mugwort sensitisation and, therefore, a higher risk to become sensitised to peach nsLTP.

Finally, the abundance of nsLTPs in plant tissues that are consumed may also play a role. For example, wheat nsLTP is abundant in the outer layers of grain, but these are removed during milling to yield the white flour that is

usually consumed in foods. Maybe this is why allergy to wheat nsLTPs does not seem to be as widely reported as that to peach.

Recent studies of allergenic plant-food nonspecific lipid-transfer proteins

Since the first reports on the identification of allergenic nsLTPs by Spanish and Italian research teams [41,42], the list of plant foods where nsLTPs were described as important allergens has considerably expanded. Allergenic plant-food nsLTPs have been recently reviewed [7•,43•] and several databases, such as www.allergen.org and www.allergome.org, publish up-to-date accounts. Sensitisation to nsLTPs that is frequently associated with systemic and severe reactions is thought to be related to the high resistance of these molecules to thermal and proteolytic degradation. A recent study has addressed the stability of the cherry nsLTP, Pru av 3, and demonstrated that its IgE reactivity was not diminished in thermally processed fruits [36•]. Walnut nsLTP, Jug r 3, was shown to provoke severe allergic reactions in an Italian population of nsLTP mono-sensitised patients [44•]. Walnut nsLTP shares IgE cross-reactivity with the nsLTP from peach, which, it was argued, was the primary sensitizer [44•]. Similarly, the hazelnut nsLTP, Cor a 8, was demonstrated as a major allergen in a Spanish group of hazelnut-allergic individuals without birch pollen allergy [45•]. In addition, the preparation and use of recombinant Cor a 8 resulted in an increased sensitivity of in-vitro diagnosis compared with hazelnut extracts. With the recombinant production of the peach lipid-transfer protein Pru p 3 in the yeast *Pichia pastoris* another useful tool for diagnosis was made available [46].

An nsLTP, Lac s 1, has been demonstrated to be a major allergen in the majority of patients suffering from the not-so-common lettuce allergy [47] and the nsLTP from grape, Vit v 1, which cross-reacts with the major peach allergen Pru p 3, was implicated in anaphylactic reactions to wine [34]. Indications that the nsLTPs from asparagus play a role in anaphylaxis and occupational asthma of individuals who frequently handle asparagus were recently reported from Spain [48]. Finally, the as yet unpublished allergenic nsLTPs from lemon, Cit l 3, and from orange, Cit s 3, have been accepted by the Allergen Nomenclature Sub-Committee of the International Union of Immunological Societies (www.allergen.org).

Recent studies of allergenic pollen and other inhalant nonspecific lipid-transfer proteins

It is obvious that allergenic nsLTPs from pollen have received less attention than their food counterparts and then mainly as potential primary sensitizers [43•]. Like the 2 S albumins, nsLTPs can sensitise both by inhalation and the oral route, since they can be found in pollen

and dusts as well as in foods. An individual has been reported with an occupational allergy to spelt who experienced symptoms on consuming a variety of fruits and where the major allergen was an nsLTP, indicating some true IgE cross-reactivity [49]. In addition to the well-known *Parietaria* allergens, nsLTPs have also been identified as allergens of several other pollens [7**] but as yet there is no clear evidence of a link between sensitisation to pollen nsLTPs and subsequent development of allergy to fruit. Mugwort has been shown to express an allergenic pollen nsLTP that cross-reacts with nsLTPs from food and in the case of chestnut induces the allergy [50]. In contrast Pastorello *et al.* [51] demonstrated that allergy to nsLTPs in food is not dependent on sensitisation to nsLTP-containing pollen. An allergenic nsLTP was also detected in the latex of *Hevea brasiliensis* as Hev b 12 which according to in-vitro data possessed cross-reactivity to nsLTPs from peach and apple [52].

Conclusion

We have still much to understand about what particular molecular properties make an nsLTP an allergen. The information being gained from a homologous series of allergens, such as the nsLTP family, will undoubtedly pave the way for an evidence-based understanding of this matter. It will be particularly interesting to correlate allergenicity and cross-reactivity with molecular structure and stability. At present, the available information allows speculations but is still too limited. nsLTPs will keep providing a subject for investigation for allergologists and structural biologists alike.

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