

Atopic allergens of plant foods

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The exact knowledge of what structural characteristics of an antigen are responsible for its allergenicity may not be available for several years. However, it is striking that the majority of the plant food allergens are clustered within a few protein families, and these are reviewed in this article. In most cases, plant food allergens are proteins that ensure the survival of the species as either seed storage proteins or proteins that are actively involved in the defence of the organism. *Curr Opin Allergy Clin Immunol* 1:261–267. © 2001 Lippincott Williams & Wilkins.

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Abbreviations

LTP	lipid transfer proteins
OAS	oral allergy syndrome
PR	pathogenesis-related proteins
TLP	thaumatin-like proteins

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Introduction

Food allergy is characteristically one of the first manifestations of the atopic syndrome and frequently affects young children. The most important allergens in the first years of life are cow's milk and hen's eggs. In the majority of cases these manifestations disappear later during childhood and are replaced by other manifestations of the atopic syndrome [1]. Allergies to plant foods are mainly seen in adults, in many cases the consequence of an allergic sensitization to pollens. IgE cross-reactivity after inhalational sensitization is believed to be the major mechanism of allergy induction in this disease. The typical symptoms can be described as oral allergy syndrome (OAS) [2], however, systemic reactions have also been observed, especially when particular foods (e.g. celery) are involved. Although the above-mentioned scenarios account for the majority of food allergy cases, in principal every food can elicit sensitization and immunologically mediated adverse reactions. One strategy to improve knowledge in the field of food allergy is to investigate the biochemistry and biology of the allergens. This is important as the physicochemical properties of the allergens determine the potency of an allergen to overcome physiological barriers such as the stomach with its acidic milieu and proteases [3]. Moreover, the classification of allergens into groups with structural similarity may help to predict cross-reactivities and thereby help to provide the food-allergic patient with useful information.

Allergens from the plant defence system

Pathogenesis-related proteins (PR) are defined as proteins that are encoded by the host plant and induced specifically in response to infections by pathogens such as fungi, bacteria, or viruses, or by adverse environmental factors. So far, PR have been classified into 14 families [4]. Many plant food allergens are homologous to proteins that are included in PR families.

Pathogenesis-related protein family 2-like allergens: beta-1,3-glucanases

Most plant beta-1,3-glucanases are endoglucanases, with the potential to partly degrade fungal cell walls by hydrolysing the beta-1,3-glucan fibres of the growing hyphae of filamentous fungi. The latex-fruit syndrome correlates allergy to *Hevea brasiliensis* latex products with allergy to certain plant foods, especially avocado, banana, chestnut, fig, and kiwi [5]. Homologous proteins present in these fruits and vegetables are responsible for the

observed cross-reactivities. The basic beta-1,3-glucanases from *Hevea* latex were recognized by specific IgE from food-allergic patients suffering from hypersensitivity to banana, potato, and tomato, rather than to latex products [6]. An abundant beta-1,3-endoglucanase was isolated from ripe bananas and its complementary DNA was cloned [7•]. Its allergenic potential remains to be determined.

Pathogenesis-related protein family 3-like allergens: class I chitinases

Almost all plant chitinases are endochitinases that degrade chitin, a major structural component of the exoskeleton of insects and of the cell walls of many pathogenic fungi. Class I chitinases contain an N-terminal so-called hevein domain with putative chitin binding properties. Relevant allergens of chestnut and avocado have been identified as class I chitinases [8]. Pers a 1, an endochitinase and major allergen from avocado was cloned and expressed in the yeast *Pichia pastoris* [9]. Hevein (Hev b 6.02) occurs in *Hevea* latex cleaved from prohevein and shares high sequence similarities with PR-3 and PR-4 proteins. Hevein contributes to the increased prevalence of fruit allergies in individuals allergic to latex. Sensitization to Pers a 1 seems to be caused by hevein in the majority of patients allergic to latex [10]. Two major IgE-binding proteins from banana were identified as class I chitinases with a hevein-like domain, further illustrating the cross-sensitization between *Hevea* latex and fruits [11].

Pathogenesis-related protein family 4-like allergens: chitinases similar to potato Win proteins

Wounding and chemical treatment of turnip plants induced the expression of an 18700 M_r allergen that was recognized by IgE of natural rubber latex allergic individuals [12]. Peptide sequences of the turnip allergen revealed 70% identity to prohevein, the Hev b 6.01 allergen from *H. brasiliensis*, and high similarities to wound-induced (Win) proteins from tomato (74%) and potato (71%), respectively.

Pathogenesis-related protein family 5-like allergens: thaumatin-like proteins

The PR-5 family comprises unique proteins with diverse functions including antifungal activity. As a result of their sequence identities with thaumatin, a sweet-tasting protein from the fruits of the west African shrub *Thaumatococcus daniellii*, these proteins are referred to as thaumatin-like proteins (TLP). TLP may also be induced by osmotic stress and are therefore also called osmotins. A 31000 M_r major apple allergen was the first TLP described as an allergen [13]. The full cDNA sequence has been obtained, and the allergen received the designation Mal d 2 from the international nomen-

clature committee (M. Krebitz, unpublished data, EMBL/Genbank accession no. AJ243427). In sweet cherry, a 23300 M_r TLP was identified as a major allergen, designated as Pru av 2, and its cDNA was cloned [14]. The N-terminal sequence of a 23000 M_r bell pepper allergen was found to be identical to a corresponding portion of the osmotin-like protein P23 from tomatoes [15].

Pathogenesis-related protein family 10-like allergens: proteins homologous to Bet v 1

The OAS, an association of food allergies to fruits, nuts, and vegetables in patients with pollen allergy, is the most frequent clinical syndrome caused by cross-reactive IgE antibodies. In the majority of cases, OAS in individuals allergic to tree pollens is caused by IgE cross-reactivity between the major birch pollen allergen Bet v 1 and its homologous proteins that have been identified in a wide range of flowering plants [16]. Bet v 1-homologous food allergens have been found in fruits of Rosaceae species (Mal d 1 in apple [17]; Pru av 1 in sweet cherry [18]; Pru ar 1 in apricot, H. Pühringer, EMBL/GenBank accession no. AF020784; D. Mbeguie-A-Mbeguie, EMBL/GenBank accession no. AF134731; Pyr c 1 in pear, F. Karamloo, EMBL/GenBank accession no. AF057030), or somatic tissues, vegetables of the Apiaceae species (Api g 1 in celery [19]; Dau c 1 in carrot [20]; pcPR1 and pcPR2 in parsley [21]). Additional Bet v 1-homologous proteins capable of binding anti-Bet v 1 IgE were described as pSTH-2 and pSTH-21 from potato [21]. The N-terminus of the major hazelnut allergen was determined and found to be 72% identical with that of the major hazel pollen allergen Cor a 1, another Bet v 1-homologue [22].

Pathogenesis-related protein family 14-like allergens: lipid transfer proteins

Plant lipid transfer proteins (LTP), named for their ability to transfer phospholipids from liposomes to mitochondria, form a family of approximately 9000 M_r polypeptides that is widely distributed throughout the plant kingdom. Most of these molecules contain eight conserved cysteines forming four disulfide bridges, which makes them highly resistant to harsh temperature and pH changes [23]. LTP are the most important allergens of peach, apricot, plum, and cherry, when no pollinosis is involved [24]. The LTP of peach was granted the designation Pru p 3 [25,26], and the LTP of apple the name Mal d 3 [27] by the international allergen nomenclature committee. The complete amino acid sequence of Pru p 3 (named Pru p 1 in that publication) has been determined [28]. IgE-mediated allergy to beer can be caused by the LTP from barley, which survives the malting and brewing process, is found intact in beer, and is involved in beer foam formation [29].

Allergens from the cupin superfamily of plant proteins: germin-like proteins, vicilins, and legumins

The term cupin (Latin *cupa*: barrel) has been given to a beta-barrel structural domain identified in a superfamily of related prokaryotic and eukaryotic proteins [30]. This protein superfamily also includes germins and germin-like proteins, and many of the seed storage proteins from higher plants.

Germin-like proteins

Wheat germin, a protease-resistant glycoprotein with oxalate oxidase activity, is the best characterized of all the cupin proteins [31]. Wheat germin and its homologues from barley and other cereals show structural similarities to other desiccation-tolerant proteins, including 7S and 11S seed storage proteins [32]. Germin-like proteins have a maximum sequence identity of approximately 90% to wheat germin, on the average closer to 50%. They are expressed at specific developmental stages in plants, are linked to plant-microbe interactions and are induced by abiotic stress in plants. An allergenic single domain germin-like protein was recently identified in ground black pepper [33].

Vicilins

The globular seed storage proteins of plants called vicilins or euvicilins (sedimentation coefficient 7S) and legumins (sedimentation coefficient 11S) are two-domain proteins. Among the vicilins or 7S globulins are two major variants in size, the regular vicilins with subunits of approximately 50 000 M_r and the large vicilins of the convicilin and beta-conglycinin type, which have additional N-terminal extensions of approximately 80–190 residues in length [34]. The 7S proteins are trimers, with each subunit being a 50 000–70 000 M_r polypeptide. Probably the best characterized member of the vicilin family is the major peanut allergen Ara h 1, which is responsible for the majority of cases of fatal food-induced anaphylaxis. The linear IgE epitopes have been mapped and shown to consist of 23 independent binding sites [35]. Mutational analysis of the immunodominant epitopes revealed that single amino acid changes within these peptides had dramatic effects on IgE-binding characteristics. The 63 500 M_r Ara h 1 forms highly stable homotrimers, a characteristic that may be important in establishing it as an allergen [36]. The majority of the IgE-binding epitopes are located in the area of the monomer-monomer contacts. These sites are protected from protease degradation, indicating that the protein structure may play a significant role in overall allergenicity [37••].

Beta-conglycinin is a major soybean globulin. It is a glycoprotein with a molecular mass of 180 000 M_r . It is a trimer that is composed of three subunits alpha, alpha',

and beta, that have molecular masses of 76 000, 72 000 and 53 000 M_r , respectively [38]. One of the major allergenic proteins in the soybean 7S-globulin fraction was identified as the alpha subunit of beta-conglycinin [39]. In addition to allergenic vicilins from peanut and soy, Jug r 2, a 47 000 M_r seed storage protein from English walnut, is the third vicilin food allergen to be identified [40].

Legumins

The legumins or 11S globulins are hexameric proteins of approximately 360 000 M_r . Each subunit consists of an acidic 30 000–40 000 M_r polypeptide linked by a disulfide bridge to a 20 000 M_r basic polypeptide. Glycinins are synthesized as approximately 60 000 M_r precursor proteins that are cleaved into the 40 000 and 20 000 M_r polypeptides of the subunits. The 14 000 M_r Ara h 3 allergen represents the N-terminal part of a 57 000 M_r peanut glycinin subunit. A cDNA clone encoding the full-length protein was isolated and linear IgE-binding epitopes of Ara h 3 were mapped [41]. The cDNA clone of Ara h 4 encodes a protein of a calculated molecular mass of 35 900 M_r , with a sequence similarity of 70% to the glycinin family of proteins [42].

The 11S fraction of soybean proteins consists almost entirely of glycinin, the principal soy globulin. Soybean glycinin is a major seed storage protein that makes up more than 20% of the soybean seed by dry weight and 35–40% of total soy protein. Native glycinin is a 350 000 M_r hexamer composed of six non-identical subunits. The five known subunits (G1–G5) of soybean glycinins are encoded by a small gene family. IgE epitopes for the soybean glycinin G1 acidic chain have been determined and found to be similar to IgE epitopes of the peanut glycinin Ara h 3 [43•]. Moreover, each basic chain from the five glycinin subunits reacted with IgE from soybean-allergic individuals to a similar extent [44]. The linear IgE epitopes of the basic chain of the glycinin G2 subunit were mapped and a three-dimensional model was developed using the known structure of the 7S phaseolin [45••].

Allergens from the prolamin superfamily of plant proteins: 2S albumin seed storage proteins, and inhibitors of alpha-amylases and proteases

Prolamins are the major storage proteins in all cereals except oats and rice. Prolamins are characterized by their solubility in alcohol/water mixtures and their high contents of proline and glutamine. The broader definition of the prolamin superfamily based on comparisons of amino acid sequences includes the 2S albumins and the closely related inhibitors of alpha-amylases and serin proteases of cereal grains.

2S albumin seed storage proteins

The 2S albumins constitute a family of structurally related homologous proteins, form a major group of storage proteins in many dicot plant species, and are members of a much wider family of proteins called the prolamin superfamily [46]. Many 2S albumins contain high levels of sulfur-containing amino acid residues. Typical 2S albumin, such as the napins from the *Brassicaceae* or the brazil nut 2S albumin, are heterodimeric proteins consisting of two subunits of approximately 4000 and 9000 M_r, which are cleaved from a single chain precursor and are held together by conserved interchain disulfide bonds. The major role of 2S albumins is as seed storage proteins for the developing seed. In addition, antifungal activity against a number of plant-pathogenic fungi has been shown for the napins from radish [47].

Allergens among the 2S albumins include Sin a 1 from yellow mustard seeds (*Sinapis alba* [48]), Bra j 1 from oriental mustard seeds (*Brassica juncea* [49]), the napin BnIII from oilseed rape (*Brassica napus* [50]), Ber e 1 from Brazil nut (*Bertholletia excelsior* [51]) Jug r 1 from the English walnut (*Juglans regia* [52]), and SFA-8/SSA from sunflower seeds (*Helianthus annuus* [53•]).

Among the peanut allergens, the 17 500 M_r glycoprotein Ara h 2 [54], the 14 500 M_r Ara h 6 [42], and the 15 800 M_r Ara h 7 [42] belong to the conglutin protein family, which is related to the 2S superfamily of seed storage proteins.

Inhibitors of alpha-amylases and proteases

Sequence homology places this group of inhibitors of cereal grains within the prolamin superfamily, which includes the 2S albumins and many prolamin storage proteins. Inhibitors of this family have been identified as allergens of cereal flours. They typically have subunits of 12 000–16 000 M_r, exist in monomeric, dimeric and tetrameric forms, and possess inhibitory activities against various types of alpha-amylases or trypsin, or are bifunctional [55]. These allergens are capable of sensitizing susceptible atopic patients by ingestion or by inhalation [56].

Allergens within the cereal superfamily of inhibitors include the glycosylated subunits of the tetrameric CM16* inhibitor from wheat [57], the homologous glycoproteins CMB* [57], Hor v 1/BMAI-1 [58], and barley dimeric protein [59] from barley, and three members of the alpha-amylase/trypsin inhibitor family from rye flour, Sec c 1 [59], RDAI-1, and RDAI-3 [60]. The 14 800 M_r major allergenic protein from rice seeds (rice allergenic protein) is another food allergen that is a member of the alpha-amylase/trypsin

inhibitor family, which shows considerable similarity to barley trypsin inhibitor and wheat alpha-amylase inhibitor [61].

Kunitz protease inhibitors

The Kunitz family of trypsin inhibitors are present in seeds of a wide range of leguminous plants, but have been characterized in most detail from soybean. Soybean trypsin inhibitor has been shown to inhibit the growth of lepidopteran and coleopteran larvae [62]. The 20 000 M_r Kunitz soybean trypsin inhibitor bound IgE of 20% of soy challenge-positive patients indicating that this protein is a minor allergen [63]. However, the Kunitz soybean trypsin inhibitor was also reported to induce food anaphylaxis [64].

Papain-like cysteine proteases

The class of thiol proteases includes several proteolytic plant enzymes, such as papain from papaya, ficin from fig, bromelain from pineapple, and actinidin from kiwi. Patients with allergic reactions to kiwi fruit produced IgE that was also reactive to bromelain and papain [65]. The cross-reactivity of papain-like allergens was further shown by cross-inhibition experiments including ficin and papain [66]. Actinidin, a thiol protease of kiwi (*Actinidia chinensis*), which accounts for approximately 50% of the soluble protein of the fruit, was identified as its major allergen and designated Act c 1 [67]. P34/Gly m Bd 30K, a soybean seed storage vacuole protein, is a plant thiol protease and an outlying member of the papain superfamily. P34, also named Gly m 1, has been identified as a major soybean allergen in patients with atopic dermatitis and food allergy [68]. The amino acid sequence of the five immunodominant epitopes was determined by sequential substitution, two of which could be mutagenized into non-IgE binding peptides [69].

Profilins

The actin cytoskeleton of a cell is composed of a network of actin filaments whose organization is regulated by a number of actin-binding proteins. One of these proteins is profilin, a 12 000–15 000 M_r monomeric actin-binding protein. The allergenic nature of profilin was first discovered by the ability of the birch pollen profilin Bet v 2 to bind IgE from birch pollen-allergic individuals [70]. Currently, profilins are well-known ubiquitous cross-reactive plant allergens [71]. The study by Valenta *et al.* [71] identified profilin as an allergen present in practically all plant cells. The term pan-allergen was coined to describe the broad area of cross-reactivity. In most cases, patients who are sensitized by pollen profilins characteristically react with a wide range of profilins from nutritive allergen sources. Profilins are involved in the celery–mugwort–spice syndrome [72].

A hazelnut profilin was identified as a relevant IgE-binding protein for a minority of pollen nut-allergic individuals [73]. Grass pollen profilin-allergic patients also reacted to homologues in celery and carrots [74]. IgE of food-hypersensitive tree pollen-allergic individuals cross-reacted with profilins from apple, pear, carrot, celery, and potato [75]. An anaphylactic reaction to lychee fruit was mediated by a profilin [76]. Furthermore, profilins were identified as causing allergic reactions to tomato [77] and to pumpkin seeds [78]. The cDNA coding for profilins from peanut [42], soybean [79], and celery [80] were cloned, produced as recombinant allergens, and designated Ara h 5, Gly m 3, and Api g 4, respectively. cDNA sequences have also been obtained for profilins from pear, Pyr c 4 (S. Scheurer, unpublished data, EMBL/GenBank database entry no. AF129424), and from golden delicious apple (S. Scheurer, unpublished data, EMBL/GenBank database entry no. AF129426).

Peroxidases and lectins

A prominent 36 000 M_r allergen was isolated from wheat flour and identified by N-terminal and internal amino acid sequences as a peroxidase [81]. A 31 000 M_r peanut agglutinin was identified as a lectin that was specifically recognized by IgE from a minority of peanut-allergic patients [82].

Conclusion

Molecular allergology will have to include another discipline in its repertoire, namely structural biology, to find an answer to the question of what makes an antigen an allergen. Structural features of a protein undoubtedly result in its capacity to become an allergen. A recent review [83••] has made a first attempt at classifying allergenic structures. However, the allergenicity of a protein depends much more on detailed structural features than mere overall structural similarities. This is well documented by the occurrence of hypoallergenic isoforms of well-known allergens that differ from their allergenic counterpart in only a few amino acid residues [84]. Ferreira *et al.* [84] showed that plant allergens consist of multiple isoforms. By testing IgE-binding capacity and the capability to activate allergen-specific T cell cultures it could be demonstrated that antibodies react with other structures of the molecule than T cell receptors. B cell epitopes were obviously influenced by few amino acids, accordingly the isoforms of Bet v 1 were subdivided into molecules with high, intermediate and low IgE-binding capacity, irrespective of their T cell antigenicity. Without doubt, the combination of allergology, molecular biology, and structural biology will produce a much clearer understanding of the processes that lead to the development of type I allergic symptoms in atopic individuals.

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