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Plant food allergens — structural and functional aspects of allergenicity

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Abstract

The three dominating plant food allergen groups belong to the prolamin and cupin superfamilies and to the family 10 of pathogenesis-related proteins. The prolamin superfamily comprises allergenic 2S albumins, nonspecific lipid transfer proteins and cereal alpha-amylase/trypsin inhibitors. These allergens have related structures and are stable to thermal processing and proteolysis. The cupin superfamily comprises the allergenic 7S and 11S globulin storage proteins from peanuts, soybean and tree nuts which are heat stable and can form immunogenicity enhancing aggregates. The Bet v 1 family of allergens includes tree pollinosis-associated food allergens with low stability which induce the symptoms of the oral allergy syndrome.

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1. Introduction

Most plant food allergens belong to only a few protein families indicating that conserved structures and biological activities play a role in determining or promoting allergenic properties. Additionally, it is becoming evident that the level of exposure and

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the properties of an allergen itself play important roles in determining the allergenic potential although the molecular basis for these effects is still not understood. From our current understanding the allergenicity of a plant food protein is determined by a sum of factors including its membership to a certain protein family, its abundance, and the stability to processing and digestion.

2. Plant food allergen protein families

2.1. *The universe of plant food allergens is actually rather small*

The Pfam protein family database (Bateman et al., 2004) is a large collection of protein families and domains. The Pfam database classifies plant protein sequences into families on the basis of sequence homology, which is related to conserved three-dimensional structures and possibly function. It has become increasingly obvious that almost all plant food allergens are either storage or defence-related proteins (Breiteneder and Radauer, 2004). Recently, the Pfam database was used to assign sequences of clinically proven food allergens to protein families (Jenkins et al., 2005). At the time of this writing the Pfam version 16.0 contained 7677 protein families. Strikingly, only three dominating plant food allergen protein families/superfamilies were identified, (1) the prolamin superfamily, (2) the cupin superfamily, and (3) the Bet v 1 family. It also became clear that the distribution of allergens into these Pfam families does not merely reflect the relative abundance of the gene families in plants. The genome sequence of the model plant species *Arabidopsis* was recently completed and was estimated to encode over 25,000 distinct proteins which can be classified into structural and metabolic proteins, storage proteins, and proteins for defence against pathogens or feeding by pests. The most common protein family encoded in the *Arabidopsis* genome was the protein kinase family. The top plant food allergen families were ranked as follows: 36th prolamin superfamily, 305th cupin superfamily, 614th Bet v 1 family (Jenkins et al., 2005). This clearly indicates that the membership to one of these families is likely to be associated with a greater potential for a protein to act as an allergen in an atopic individual.

2.2. *Prolamin superfamily*

The prolamin superfamily comprises three major groups of plant food allergens, 2S albumins, nonspecific lipid transfer proteins (nsLTPs) and cereal alpha-amylase/trypsin inhibitors (Breiteneder and Radauer, 2004; Mills et al., 2004). All of these low-molecular proteins are cysteine rich, have similar three-dimensional folds that are rich in alpha-helices, and are stable to thermal processing and proteolysis. The 2S albumins are a major group of storage proteins present in many dicotyledonous plants. They include major allergens from tree nuts and seeds such as Brazil nut, walnut, sesame, and mustard. The nsLTPs play an important role in plant defence against fungi and bacteria (Blein et al., 2002). They have a wide distribution in fruits, nuts, seeds and vegetables and have attracted much interest for being highly important allergens for almost exclusively Mediterranean atopic populations. The family of cereal alpha-amylase and protease

inhibitors mediates a certain degree of resistance to insect pests that feed on plant tissues. This protein family comprises allergenic members that are produced in wheat, barley, rice, and corn. Just like the 2S albumins and the nsLTPs, the members of this protein family are capable to sensitise susceptible atopic individuals through ingestion or inhalation.

2.3. *Cupin superfamily*

The cupins are a functionally diverse superfamily of proteins that share a beta-barrel structural core domain to which the term cupin (Latin *cupa*, barrel) was given. The cupin superfamily comprises the major globulin storage proteins mainly from legumes and nuts. The globulins are divided into the 7S vicilin-like globulins and the 11S legumin-like globulins. Globulins have been found to be highly relevant allergens in plant foods including peanuts, soybean, lentils, walnut, hazelnut, and sesame (Breiteneder and Radauer, 2004; Mills et al., 2004). Despite having very low levels of sequence identity, members of the cupin superfamily have highly conserved structures. In contrast to the Bet v 1 family of plant food allergens, there is little evidence of IgE cross-reactivity between cupin allergens with an overall sequence identity of under 40%. This results in a very limited cross-reactivity between cupins from even closely related species such as peanut and pea (Wensing et al., 2003).

2.4. *Bet v 1 family*

Individuals with pollen allergy frequently suffer from allergic symptoms after eating certain plant foods. The majority of these reactions are caused by allergens of Rosaceae fruits (eg, apple, cherry, apricot, pear) and Apiaceae vegetables (eg, celery, carrot) which cross-react with allergens that are present in birch pollen, particularly the major birch pollen allergen Bet v 1 (Breiteneder and Radauer, 2004). Bet v 1 was the first of many allergens published that showed homology to family 10 of the pathogenesis-related proteins. Bet v 1-type allergens are rather unstable to heating and digestion. Consequently symptoms are mostly restricted to the oral cavity. In general, Bet v 1 from birch pollen acts as the primary sensitising agent (Vieths et al., 2002). The overall high levels of conserved surface residues between the members of the Bet v 1 family play an important role in conservation of IgE-binding epitopes and underlie the fruit–vegetable–pollen cross-reactive syndromes (Jenkins et al., 2005).

3. Molecular properties of proteins — how they might affect allergenicity

3.1. *Abundance*

Seeds and nuts contain storage proteins which may account for 50% or more of the total proteins in the organ. Most major food allergens that sensitise via the gastro-intestinal tract are present in at least 1% of the total protein content of plant foods. However, some proteins which are present in all plants in very large quantities such as the enzyme ribulose-1,5-bisphosphate carboxylase — it accounts for 30–40% of total leaf protein —

have never been reported as allergens. In contrast, nsLTPs are potent allergens but not very abundant. Thus, the amount of protein alone does not explain its allergenicity. While abundance is an important factor, it is probably secondary to protein stability.

3.2. Stability to processing and digestion

A compact three-dimensional structure, ligand-binding, disulphide bonds, and glycosylation contribute to protein stability (Breiteneder and Mills, 2005). These factors are relevant to both the resistance of proteins to denaturation by food processing and the harsh conditions of the gastro-intestinal tract. Ligand-binding can have the overall effect of reducing mobility of the polypeptide backbone, increasing both thermal stability and resistance to proteolysis. Some proteins form a cavity while others possess a tunnel into which ligands fit. nsLTPs, which possess a lipid-binding pocket, show increased stability when the pocket is occupied by fatty acids or phospholipid molecules. Bet v 1 and Bet v 1-homologous food allergens have been shown to bind steroid ligands. One of the structural features clearly related to stability is the presence of disulphide bonds. Both inter- and intrachain disulphide bridges constrain the three-dimensional fold such that perturbation of the structure by heat or chemicals is limited and frequently reversible. Important plant food allergens that have high numbers of disulphide bonds include members of the prolamin superfamily (nsLTPs, 2S albumins, cereal alpha-amylase/trypsin inhibitors) as well as of the pathogenesis-related proteins (class I chitinases, thaumatin-like proteins). N-glycosylation can have a significant stabilizing effect on protein structure. There is evidence that it increases the stability of the 7S globulin of peas and its resistance to chemical denaturation.

3.3. Interaction with lipid structures and aggregation

Many plant food allergens are able to associate with cell membranes or other types of lipid structures found in food or show a propensity to aggregate as a result of food processing (Breiteneder and Mills, 2005). The allergenic 2S albumin from mustard was shown to interact with phospholipid vesicles. This led to the proposition that such interactions might affect the uptake and processing of the allergen in the gastro-intestinal tract indicating that the biologic activity of these proteins plays a role in attenuating their allergenic potential. Similarly, there is emerging evidence that nsLTPs are also able to interact with lipid structures. A propensity of certain proteins to aggregate might affect their ability to sensitise by generally enhancing their immunogenicity. Both 7S and 11S globulins are highly thermostable. It seems that the cupin barrel remains intact but the unfolding of other regions of the protein results in a loss of structure leading to formation of large aggregates as was in detail examined for soybean globulins. Peanuts are often subjected to thermal processing at low water levels such as roasting. Thus peanut proteins become more thermostable in low water systems while at the same time glycation reactions cross-link individual molecules and increase their allergenic activity. Interaction with lipids and the formation of larger aggregates contribute to the allergenicity of plant food proteins in conjunction with the amount of protein ingested and the stability to processing and digestion.

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