

Structure, Allergenicity, and Cross-Reactivity of Plant Allergens

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Abstract Within the last two decades, hundreds of allergenic proteins from diverse sources have been identified. The availability of numerous allergen sequences provided the basis for classifying allergens into families of evolutionary and structurally related proteins. This chapter gives an introduction into the protein family classification of allergens of plant origin. Our analysis showed that the majority of plant allergens could be grouped into a small number of protein families. The most important families of plant allergens are the prolamin superfamily, which contains several groups of important food and respiratory allergens, the profilins and the Bet v 1-related proteins, two families of allergens responsible for cross-reactivity between pollen and plant foods, and the seed storage globulins, major allergenic components of legumes, tree nuts, and other dicotyledonous seeds. The molecular classification of allergens can be used to establish a correlation between sequence and structural similarity and cross-reactivity among homologous allergens, delineate common properties of allergens, and deduce possible factors that make proteins allergenic.

Keywords Food allergy · Pollen allergy · Allergens · Protein families

Abbreviations HMM – hidden Markov model; IgE – immunoglobulin; nsLTP – non-specific lipid transfer protein; PR-10 – pathogenesis-related proteins family 10.

1 Introduction

Identification and characterization of allergens is one of the major research areas in molecular allergology. Allergens are molecules that are capable of eliciting an allergic immune response in humans, which is characterized by an excess of

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T helper 2 cells that trigger the differentiation of B cells into immunoglobulin E (IgE) producing plasma cells. Most allergens are proteins. During the last two decades, hundreds of allergenic proteins from all types of sources (pollen, mites, molds, animal dander, insect venoms, natural rubber latex, and foods) have been identified and their sequences, physicochemical properties, and clinical data made available in various allergen databases (Mari 2005) such as the Official List of Allergens (<http://www.allergen.org>) and the Allergome (<http://www.allergome.org>). Most of these databases classify allergens mainly by source. However, the growing number of available allergen sequences and structures made it possible to establish a more natural classification scheme that identifies the protein families of allergens based on sequence and structural similarity.

This chapter provides the application of protein family classification to plant allergens. Our classification scheme provides a framework for discussing the common molecular properties of allergens and assessing the connection between sequence and structural similarity and cross-reactivity.

2 Protein Families of Plant Allergens

Since the 1970s, a large number of protein family databases, which use different methods for grouping proteins, have been developed (Redfern, Grant, Maibaum, and Orengo 2005). They classify proteins either by common structures (SCOP, CATH) or based on similar sequences using conserved motifs (PROSITE, PRINTS), position-specific scoring matrices (ProDom), or profile hidden Markov models (Pfam, SMART) for aligning new sequences with existing protein families.

The Pfam database (<http://Pfam.sanger.ac.uk>) is a semi-automatic collection of multiple sequence alignments and profile hidden Markov models (HMMs), which contains in its version 20.0, released in May 2006, 8296 families that cover 74% of all protein sequences in the Uniprot database (Finn, Mistry, Schuster-Bockler, Griffiths-Jones, Hollich, Lassmann, Moxon, Marshall, Khanna, Durbin, Eddy, Sonnhammer, and Bateman 2006).

We chose Pfam as a resource for the classification of allergens for several reasons: (i) sequence alignments serving as starting points for HMM calculations are manually compiled and hence more reliable than automatically generated family definitions, (ii) protein families in Pfam are defined based on sequence rather than structural similarity, which makes this database quite comprehensive, and (iii) sequence comparison using profile HMMs is more sensitive than classical sequence alignment algorithms, thus enabling the grouping of proteins sharing minimal portions of their sequences.

The search for plant allergen sequences in the Allergome database, one of the currently most comprehensive collections of allergen data (Mari 2005), yielded 321 complete or almost complete sequences (excluding variant and allelic sequences from the same source). These sequences were compared to

the Pfam database to generate a list of Pfam families and Pfam architectures of plant allergens. The Pfam architecture represents the domain organization of a protein and was chosen as the criterion for classifying allergens in order to avoid ambiguities concerning multi-domain proteins. Of the 321 plant allergen sequences, 315 were distributed among 62 Pfam architectures (Table 1) and 66 Pfam families; these are only 0.8% of all protein families in the Pfam database. In comparison, all seed plant proteins in the Uniprot database were classified into 2,615 Pfam families (Radauer and Breiteneder 2006a). The narrow distribution of plant allergens in the protein family space is highlighted by the fact that the 17 most populated allergen families contain 75% of all allergen sequences (Table 1). In comparison, the 17 most abundant families of seed plant proteins comprise only 25% of all seed plant proteins.

In addition, the protein family distribution of plant allergens does not match the distribution of protein families among all plant proteins. The prolamin superfamily, the family containing the greatest number of plant allergens, on 23rd position in the ranking of all plant protein families, is the only allergen-containing protein family ranked among the top 25 (Radauer and Breiteneder 2006a).

The two most important types of sources of plant-derived allergens are pollen and plant foods. The distribution of allergens among protein families shows significant differences between these types of sources (Fig. 1). While some allergen families are confined to certain sources such as seed storage globulins in plant foods and polcalcins in pollen, other families are found in

Table 1 The most abundant protein families of plant allergens

Protein family name	Pfam architecture	Number
Prolamin superfamily	PF00234	52
Profilin	PF00235	42
Bet v 1-related	PF00407	21
Seed storage globulin	PF00190-PF00190	20
Group 2 grass pollen allergen	PF01357	11
Thaumatococcus-like	PF00314	11
Polcalcin	PF00036-PF00036	11
Expansin	PF03330-PF01357	10
Pectate lyase	PF00544	9
Group 5 grass pollen allergen	PF01620	9
Ole e 1-related	PF01190	8
Polygalacturonase	PF00295	7
Papain-like protease	PF08246-PF00112	6
Berberine bridge enzyme	PF01565-PF08031	6
Class I chitinase	PF00187-PF00182	6
Ribosome inactivating protein	PF00161	6
Beta-1,3-glucanase	PF00332	5
Members of 45 allergen families with less than 5 members		75
Members of uncharacterized families		6

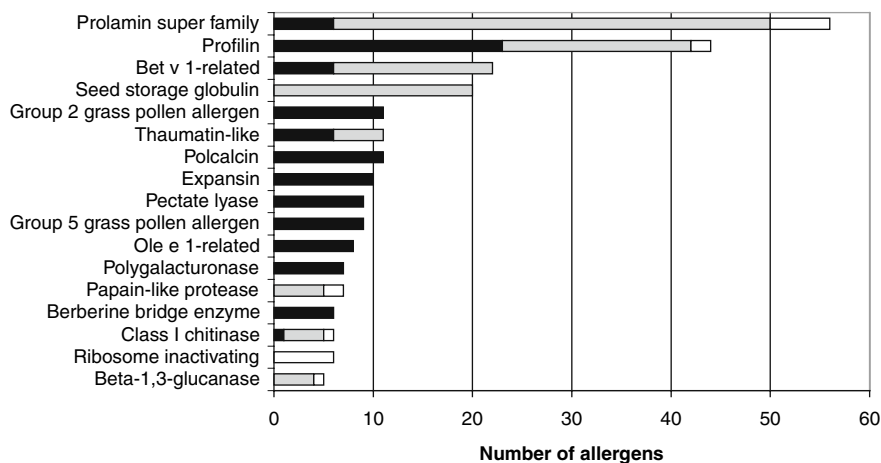


Fig. 1 Source distribution of plant allergens. *Black*: pollen, *grey*: food, *white*: other (latex and non-pollen inhalative allergens)

all types of allergen sources. Cross-reactivity among members of some of these ubiquitous allergen families, for example, profilins and Bet v 1-related allergens, are responsible for the well-known pollen and latex-associated food allergy syndromes (Vieths, Scheurer, and Ballmer-Weber 2002).

In the following sections, we describe the biochemical properties, species distributions, and allergological significance of some of the most important plant allergen families.

2.1 The Prolamin Superfamily

The existence of the prolamin superfamily was proposed as early as 1985 (Kreis, Forde, Rahman, Mifflin, and Shewry 1985), and it derives its name from the alcohol-soluble proline and glutamine-rich storage proteins of cereals such as wheat, barley, and rye, but not rice. Although allergic reactions to prolamins from wheat have been reported (Sandiford, Tatham, Fido, Welch, Jones, Tee, Shewry, and Newman Taylor 1997), the major allergens that are thought to sensitize atopic individuals through the gastrointestinal tract belong to one of the three other related protein families of this superfamily. These are the 2S albumins, the non-specific lipid transfer proteins (nsLTPs), and the cereal inhibitors of alpha-amylase and/or trypsin (Mills, Jenkins, Alcocer, and Shewry 2004). Like all members of the prolamin superfamily, these proteins are characterized by the presence of a conserved pattern of six or eight cysteine residues that form three or four intramolecular disulfide bonds. Disulfide bonds are a molecular feature that conveys stability to heating and digestion to these molecules, an important characteristic of true food allergens (Breiteneder and

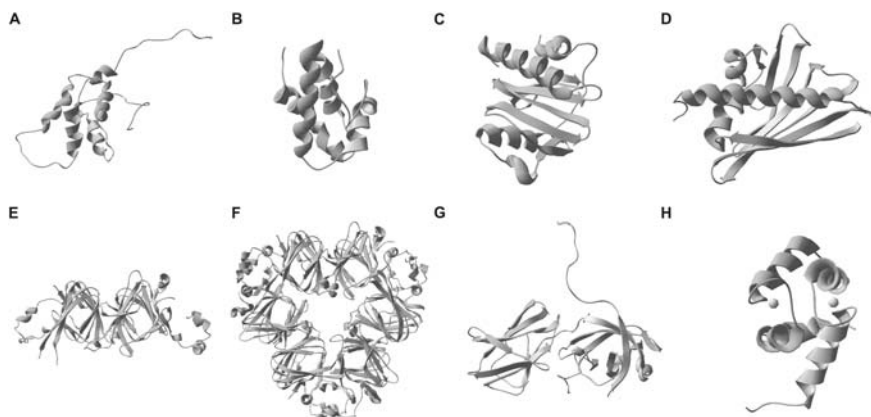


Fig. 2 Structures of the members of important plant allergen families. A: Ara h 6, a 2S albumin-related allergen from peanut (PDB accession number 1W2Q), B: Pru p 3, the non-specific lipid transfer protein from peach (2ALG), C: *A. thaliana* pollen profilin (1A0K), D: Bet v 1 from birch pollen (1BV1), E, F: single subunit and trimer of soybean β -conglycinin (1FXZ and 1OD5), G: Phl p 1, a β -expansin from timothy grass pollen (1N10), H: Bet v 4, a polcalcin from birch pollen (1H4B)

Mills 2005c). All of these low-molecular-weight proteins have a related architecture that consists of bundles of four α -helices stabilized by their intra-chain disulfide bonds (Fig. 2A, B). Interestingly, the order of the disulfide bonds in the nsLTPs is different from the 2S albumins or the bifunctional inhibitors (Mills et al. 2004).

The 2S albumins are a major group of seed storage proteins from a botanically diverse range of dicotyledonous plants. These include not only economically important temperate crops such as oilseed rape, mustard, sunflower, lupine, and pea, but also crops from warmer climates such as sesame and peanut. Apart from their function as storage proteins, activities as inhibitors of proteinases have been reported for 2S albumins from the brassicas *Sinapis arvensis* (Svendsen, Nicolova, Goshev, and Genov 1994) and *Brassica nigra* (Genov, Goshev, Nikolova, Georgieva, Filippi, and Svendsen 1997), and an antifungal activity has been described for a 2S albumin from radish (*Raphanus sativa*) (Terras, Schoofs, De Bolle, Van Leuven, Rees, Vanderleyden, Cammue, and Broekaert 1992).

Many of the seed and tree nut allergens belong to the 2S albumins. The best characterized allergenic 2S albumin is probably the one from yellow mustard, Sin a 1 (Menendez-Arias, Moneo, Dominguez, and Rodriguez 1988). Other allergenic 2S albumins are Ber e 1 from Brazil nut (Alcocer, Murtagh, Bailey, Dumoulin, Meseguer, Parker, and Archer 2002), Jug r 1 from English walnut (Teuber, Dandekar, Peterson, and Sellers 1998), and Ana 3 from cashew nut (Teuber, Sathe, Peterson, and Roux 2002). The allergens Ara h 2 and Ara h 6 from peanut are members of the conglutin family, which is closely related to the 2S albumin family (Kleber-Janke, Crameri, Appenzeller, Schlaak, and Becker

1999). Recently, a high-resolution structure of Ara h 6 (Fig. 2A) became available (Lehmann, Schweimer, Reese, Randow, Suhr, Becker, Vieths, and Rosch 2006). Additional allergenic 2S albumins have been identified in chickpea (Vioque, Sanchez-Vioque, Clemente, Pedroche, Bautista, and Millan 1999), sunflower (Kelly and Hefle 2000), almond (Poltronieri, Cappello, Dohmae, Conti, Fortunato, Pastorello, Ortolani, and Zacheo 2002), and sesame seed (Pastorello, Varin, Farioli, Pravettoni, Ortolani, Trambaioli, Fortunato, Giuffrida, Rivolta, Robino, Calamari, Lacava, and Conti 2001).

The family of nsLTPs is not restricted to seed tissues as the 2S albumins, but is frequently found in the epidermal tissues of fruits. Comparisons of their sequences show that the nsLTPs are closely homologous basic proteins. All nsLTPs possess eight conserved cysteines and are stabilized by four intra-chain disulfide bonds. nsLTPs have been well defined at a structural level, with the first structure being defined for corn (Shin, Lee, Hwang, Kim, and Suh 1995) and the most recent one for peach (Pasquato, Berni, Folli, Folloni, Cianci, Pantano, Helliwell, and Zanotti 2006), shown in Fig. 2B. All nsLTPs are characterized by a related fold consisting of bundles of four α -helices stabilized by disulfide bonds with a central hydrophobic tunnel into which lipophilic molecules can bind (Breiteneder and Mills 2005b). nsLTPs have been suggested to mediate the transfer of phospholipids between vesicles and membranes (Guerbette, Grosbois, Jolliot-Croquin, Kader, and Zachowski 1999). However, it is now becoming clear 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 (Tassin, Broekaert, Marion, Acland, Ptak, Vovelle, and Sodano 1998). Instead, lipid transfer proteins may play an important role in plant defense against fungi and bacteria, the exact mechanism of action still being unknown (Cheng, Samuel, Liu, Shyu, Lai, Lin, and Lyu 2004).

Allergenic nsLTPs are widely distributed, with sequences available from fruits, nuts, seeds, vegetables, pollen, and *Hevea brasiliensis* latex. The peach nsLTP, Pru p 3, was the first to be characterized (Pastorello, Farioli, Pravettoni, Ortolani, Ispano, Monza, Baroglio, Scibola, Ansaloni, Incorvaia, and Conti 1999; Sanchez-Monge, Lombardero, Garcia-Selles, Barber, and Salcedo 1999). Recent reviews have summarized the current clinical and molecular knowledge of allergenic plant food (Pastorello and Robino 2004; Salcedo, Sanchez-Monge, Diaz-Perales, Garcia-Casado, and Barber 2004) and pollen nsLTPs (Breiteneder and Mills 2005a; Weber 2005).

Seeds of wheat, barley, rye, corn, and rice contain a family of inhibitors of trypsin and non-plant alpha amylases. These inhibitors interfere with the digestion of plant starches and proteins by impeding insect gut enzymes. They possess eight, nine, or ten cysteine residues that form four or five intra-chain disulfide bridges. Allergens belonging to the cereal inhibitor family are involved in both respiratory and food allergies (James, Sixbey, Helm, Bannon, and Burks 1997; Pastorello et al. 1999). Members of this family of proteins exist as monomers, dimers, or tetramers, with the most active containing glycosylated

subunits in a monomeric or tetrameric form. In barley these subunits were designated BMAI-1 (Mena, Sanchez-Monge, Gomez, Salcedo, and Carbonero 1992), now Hor v 15, and BTAI-CMb (Sanchez-Monge, Gomez, Barber, Lopez-Otin, Armentia, and Salcedo 1992), and in wheat WTAI-CM16 (Sanchez-Monge et al. 1992). The other well-characterized allergenic cereal inhibitor family is from rice. RDAI-1 and RDAI-3 are rice dimeric alpha amylase inhibitors (Nakase, Adachi, Urisu, Miyashita, Alvarez, Nagasaka, Aoki, Nakamura, and Matsuda 1996; Salcedo, Diaz-Perales, and Sanchez-Monge 2001).

2.2 Profilins

Profilins are cytosolic proteins of 12–15 kDa in size, which are found in all eukaryotic cells. Profilins bind to monomeric actin (G actin) and a plethora of other proteins, thus regulating the dynamics of actin polymerization during processes such as cell movement, cytokinesis, and signaling (Witke 2004).

Profilins from higher plants constitute a family of highly conserved proteins showing sequence identities of at least 75% even between members from distantly related organisms (Radauer, Willeroider, Fuchs, Hoffmann-Sommergruber, Thalhamer, Ferreira, Scheiner, and Breiteneder 2006b). Plant profilins were originally discovered as cross-reactive pollen allergens eliciting IgE responses in 10–20% of pollen-allergic patients. Later, they were also described as allergens in plant foods and *Hevea* latex (reviewed in Radauer and Hoffmann-Sommergruber 2004). Since profilin-specific IgE usually cross-reacts with homologs from virtually every plant source (Radauer et al. 2006b), sensitization to these allergens has been considered a risk factor for allergic reactions to multiple pollen sources (Mari 2001) and for pollen-associated food allergy (Asero, Mistrello, Roncarolo, Amato, Zanoni, Barocci, and Caldironi 2003). However, the clinical relevance of profilin-specific IgE has frequently been questioned (Wensing, Akkerdaas, van Leeuwen, Stapel, Bruijnzeel-Koomen, Aalberse, Bast, Knulst, and van Ree 2002).

Structures of three plant profilins have been published: those from *Arabidopsis thaliana* pollen (Thorn, Christensen, Shigeta, Huddler, Shalaby, Lindberg, Chua, and Schutt 1997), birch pollen (Fedorov, Ball, Mahoney, Valenta, and Almo 1997), and *H. brasiliensis* latex (PDB accession number 1G5U). Profilins fold into globular structures consisting of a central seven-stranded anti-parallel β -sheet and two α -helices each on either side (Fig. 2C). Despite sequence identities of below 30%, plant profilin structures are highly similar to the structures of profilins from mammals, fungi, and amoeba. This explains the finding that IgE directed to plant profilins weakly binds to the human homolog as well (Valenta, Duchene, Pettenburger, Sillaber, Valent, Bettelheim, Breitenbach, Rumpold, Kraft, and Scheiner 1991). However, no profilins from sources other than plants have been shown to elicit allergic reactions.

2.3 *Bet v 1-Related Allergens*

The identification of the major birch pollen allergen Bet v 1 was published in 1989 (Breiteneder, Pettenburger, Bito, Valenta, Kraft, Rumpold, Scheiner, and Breitenbach 1989). Since then, this allergen has become the most intensely studied allergen worldwide. Bet v 1 is a PR-10 (pathogenesis-related proteins family 10) like protein. Apart from pathogenesis-related proteins and Bet v 1-related allergens, the PR-10 family contains the family of major latex proteins, named after a major component of the latex of opium poppy, as well as several enzymes involved in secondary plant metabolism (Liscombe, MacLeod, Loukanina, Nandi, and Facchini 2005). PR-10 proteins fold into structures consisting of a seven-stranded anti-parallel β -sheet wrapped around a long C-terminal α -helix and two small N-terminal helices (Fig. 2D). The crystal structure of the naturally occurring isoform Bet v 1 I was recently determined in complex with deoxycholate (Markovic-Housley, Degano, Lamba, von Roepenack-Lahaye, Clemens, Susani, Ferreira, Scheiner, and Breiteneder 2003). In addition, ligand-binding studies with Bet v 1 I (Markovic-Housley et al. 2003), Bet v 1.2801 (Mogensen, Wimmer, Larsen, Spangfort, and Otzen 2002), and with the cherry homolog Pru av 1 (Neudecker, Schweimer, Nerkamp, Scheurer, Vieths, Sticht, and Rösch 2001) showed interaction with various phytosteroids. Therefore, it was suggested that Bet v 1 and related PR-10 proteins might function as plant steroid carriers (Markovic-Housley et al. 2003).

Bet v 1-related tree pollen allergens are limited to the botanical order of the Fagales; they include Cor a 1 from hazel, Aln g 1 from alder, Car b 1 from hornbeam and major allergens from other Fagales trees. On the other hand, cross-reactive Bet v 1 homologs have been identified from fruits of the Rosaceae family, e.g., Mal d 1 from apple (Vanek-Krebitz, Hoffmann-Sommergruber, Laimer da Camara Machado, Susani, Ebner, Kraft, Scheiner, and Breiteneder 1995), Pru av 1 from cherry (Scheurer, Metzner, Haustein, and Vieths 1997), and Pyr c 1 from pear (Karamloo, Scheurer, Wangorsch, May, Haustein, and Vieths 2001). Furthermore, Bet v 1-related allergens are found in vegetables from the Apiaceae family, such as Api g 1 from celery (Breiteneder, Hoffmann-Sommergruber, O'Riordain, Susani, Ahorn, Ebner, Kraft, and Scheiner 1995) and Dau c 1 from carrot (Hoffmann-Sommergruber, O'Riordain, Ahorn, Ebner, Laimer Da Camara Machado, Pühringer, Scheiner, and Breiteneder 1999), as well as from the legume seeds peanut (Mittag, Akkerdaas, Ballmer-Weber, Vogel, Wensing, Becker, Koppelman, Knulst, Helbling, Hefle, Van Ree, and Vieths 2004), soybean (Kleine-Tebbe, Vogel, Crowell, Haustein, and Vieths 2002), and mung bean (Mittag, Vieths, Vogel, Wagner-Loew, Starke, Hunziker, Becker, and Ballmer-Weber 2005). The presence of high concentrations of Bet v 1-related allergens in these foods is the explanation for the high frequency of allergic reactions to plant foods among birch pollen-allergic patients (Vieths et al. 2002).

2.4 Seed Storage Globulins

Seed storage globulins are classified into two groups based on their sedimentation coefficients. The 7/8 S globulins occur in a range of monocot and dicot plant species and are often referred to as vicilins. The 11S-12S globulins are the most widespread group and are present in monocot and dicot seeds, particularly legume seeds, hence they are often called legumins. Both vicilins and legumins belong to the cupin superfamily. The cupins are a large and functionally immensely diverse superfamily of proteins (Dunwell, Purvis, and Khuri 2004) that have a common origin and whose evolution can be followed from bacteria to eukaryotes including animals and higher plants (Dunwell 1998; Dunwell, Khuri, and Gane 2000). These proteins share a beta-barrel structural core domain to which the term cupin (derived from the Latin word *cupa* for barrel) was given (Fig. 2E). Cupins can be divided into single-domain cupins and two-domain bicupins, which are thought to have evolved from the duplication of a microbial sequence. Bicupins include the globulin seed storage proteins that constitute major components of the human diet. Globulins are highly abundant in nuts and seeds, comprising up to 70% of the seed protein.

Mature 7/8S globulins are homotrimeric proteins of about 150–190 kDa. Presently, the three-dimensional structures of four 7/8S globulins have been solved, namely canavalin from jack bean (Ko, Day, and McPherson 2000; Ko, Kuznetsov, Malkin, Day, and McPherson 2001), phaseolin from French bean (Lawrence, Izard, Beuchat, Blagrove, and Colman 1994), the beta subunit of beta-conglycinin from soybean (Maruyama, Adachi, Takahashi, Yagasaki, Kohno, Takenaka, Okuda, Nakagawa, Mikami, and Utsumi 2001), and the 8S α globulin from mung bean (Itoh, Garcia, Adachi, Maruyama, Tecson-Mendoza, Mikami, and Utsumi 2006). These structures illustrate that trimeric vicilins are disc shaped (Fig. 2E, F). Vicilins lack cysteines and, therefore, contain no disulfide bonds. Their detailed subunit compositions vary considerably due to the differences in proteolytic processing and glycosylation of the monomers (Shewry, Napier, and Tatham 1995).

The best-analyzed allergenic vicilin is most likely the major peanut allergen Ara h 1 that is responsible for the majority of cases of fatal anaphylaxis induced by a plant food. Ara h 1 is one of the main seed storage proteins of peanut and is recognized by serum IgE from over 90% of peanut allergic individuals (Burks, Williams, Helm, Connaughton, Cockrell, and O'Brien 1991). Various studies have confirmed the allergenic activity of the 7S globulins Jug r 2 from walnut (Teuber, Jarvis, Dandekar, Peterson, and Ansari 1999), Ana o 2 from cashew nut (Wang, Robotham, Teuber, Tawde, Sathe, and Roux 2002), Ses i 3 from sesame (Beyer, Bardina, Grishina, and Sampson 2002), Len c 1 from lentil (Lopez-Torrejon, Salcedo, Martin-Esteban, Diaz-Perales, Pascual, and Sanchez-Monge 2003), Pis s 1 from pea (Sanchez-Monge, Lopez-Torrejon, Pascual, Varela, Martin-Esteban, and Salcedo 2004), and Cor a 11 from

hazelnut (Lauer, Foetisch, Kolarich, Ballmer-Weber, Conti, Altmann, Vieths, and Scheurer 2004).

Mature 11S globulins are hexameric proteins that are initially assembled and transported through the secretory system as intermediate trimers (Shewry et al. 1995). In the protein storage vacuole, the subunits of the trimers are proteolytically processed to yield an acidic 30–40 kDa polypeptide linked by a disulfide bond to a basic polypeptide of ~20 kDa. Cleavage is accompanied by the transformation of two trimers into a mature hexameric 11S globulin (Müntz 1998). The three-dimensional structures of the trimeric precursor and the hexameric mature protein of glycinin from soybean have been determined (Adachi, Kanamori, Masuda, Yagasaki, Kitamura, Mikami, and Utsumi 2003; Adachi, Takenaka, Gidamis, Mikami, and Utsumi 2001).

The minor peanut allergen Ara h 3 was identified as the N-terminal portion of a peanut glycinin subunit (Rabjohn, Helm, Stanley, West, Sampson, Burks, and Bannon 1999). The 11S fraction of soybean proteins consists almost entirely of glycinin, the predominant soy storage globulin. Native glycinin is a 350-kDa hexamer composed of different combinations of the five subunits G1 to G5. IgE epitopes of the acidic chain of the soybean glycinin subunit G1 have been found to be similar to IgE-epitopes of the peanut glycinin Ara h 3 (Beardslee, Zeece, Sarath, and Markwell 2000). Each basic chain of the five soybean glycinin subunits reacted with IgE from soybean allergic individuals to a similar extent (Helm, Cockrell, Connaughton, Sampson, Bannon, Beilinson, Livingstone, Nielsen, and Burks 2000). Additional allergenic 11S legumins are Ber e 2 from Brazil nut (Bartolome, Mendez, Armentia, Vallverdu, and Palacios 1997), Ses i 6 from sesame (Tai, Wu, Chen, and Tzen 1999), and Fag e 1 from buckwheat (Fujino, Funatsuki, Inada, Shimono, and Kikuta 2001).

2.5 Expansins and Expansin-Related Allergens

Expansins are ubiquitous plant cell wall glycoproteins that catalyze cell wall loosening during cell growth and other developmental processes (reviewed in Sampedro and Cosgrove 2005). They are typically 250–275 amino acids long. Phylogenetic analysis revealed the existence of four subfamilies designated α -expansins, β -expansins, expansin-like A, and expansin-like B. Sequence identity between members of different subfamilies is only 20–40%.

Expansin structures consist of two domains preceded by a poorly conserved unstructured glycosylated N-terminal stretch (Fig. 2G). The N-terminal domain folds into a six-stranded β -barrel stabilized by three disulfide bonds (Fig. 2G, right domain). It is distantly related and structurally similar to glycoside hydrolase family 45 proteins. Despite the conservation of several residues that form the active center in those enzymes, no hydrolytic activity has been detected for expansins. Instead, they exert their cell wall loosening activity by breaking non-covalent bonds between polysaccharide molecules (Cosgrove

2000). The C-terminal expansin domain adopts an immunoglobulin-like two-layered β -sandwich structure (Fig. 2G, left domain).

Several groups of allergens are related to one or both expansin domains. Group 1 grass pollen allergens are β -expansins. They are the major allergens recognized by IgE from 90% of grass pollen allergic patients (Andersson and Lidholm 2003). Interestingly, no expansins outside the grass family have been shown to be allergenic. The groups 2 and 3 of grass pollen allergens are related to β -expansins showing 35–45% sequence identities. However, they lack the N-terminal domain and are thought to have evolved from a truncated β -expansin gene. Unlike group 1 grass pollen allergens, members of the groups 2 and 3 show no cell wall loosening activity and their biologic activity is unknown. These proteins are minor allergens and show only weak cross-reactivity with group 1 allergens (Andersson et al. 2003).

Recently, kiwellin, a cell wall protein from kiwi fruits, has been sequenced and identified as an allergen (Tamburrini, Cerasuolo, Carratore, Stanziola, Zofra, Romano, Camardella, and Ciardiello 2005). The protein is similar to ripening-related proteins from grape, potato, and rice. Furthermore, it is distantly related to the N-terminal domain of α -expansins, with only 20–25% sequence identity. Another protein family with weak homology to the N-terminal expansin domain (sequence identities of 20–30%) is the barwin family, also termed pathogenesis-related proteins PR-4, a widespread family of proteins expressed upon wounding or pathogen attack in plants (Neuhaus 1999). Barwin domains are found as the C-terminal parts of the major latex allergen Hev b 6 (prohevein) and the turnip allergen Bra r 2 (Breiteneder and Radauer 2004). All these proteins are members of a superfamily of diverse proteins sharing a double-psi β -barrel as the common structural motif (Castillo, Mizuguchi, Dhanaraj, Albert, Blundell, and Murzin 1999).

2.6 Polcalcins

Polcalcins are cytosolic proteins with molecular masses of 9 kDa. They contain two calcium-binding EF hand motifs (Fig. 2H). The EF hand is a ubiquitous helix-loop-helix motif found in a large number of calcium-binding proteins (Grabarek 2006). Polcalcins are specifically expressed in anthers and pollen, their biological function still being unknown (Okada, Sasaki, Ohta, Onozuka, and Toriyama 2000).

Polcalcins are minor allergens causing IgE reactivity in 10–45% of pollen-allergic patients. Allergenic polcalcins were described in plants from the families Poaceae (timothy, Bermuda grass), Oleaceae (olive, lilac), Brassicaceae (oilseed rape, turnip), Betulaceae (birch, alder), and Chenopodiaceae (white goosefoot). As members from different plant families show at least 65% sequence identity, cross-reactivity among polcalcins is considerably high (Ledesma, Barderas,

Westritschnig, Quiralte, Pascual, Valenta, Villalba, and Rodriguez 2006; Tinghino, Twardosz, Barletta, Puggioni, Iacovacci, Butteroni, Afferni, Mari, Hayek, Di Felice, Focke, Westritschnig, Valenta, and Pini 2002). Structures of three polcalcins were solved. While the allergens from timothy (Phl p 7) and goosefoot (Che a 3) pollen fold into a domain-swapped dimer (Verdino, Westritschnig, Valenta, and Keller 2002), the structure of Bet v 4 from birch pollen (Fig. 2H) was shown to be monomeric (Neudecker, Nerkamp, Eisenmann, Nourse, Lauber, Schweimer, Lehmann, Schwarzing, Ferreira, and Rösch 2004). Structural changes accompanying binding of calcium in all EF hand proteins explain the loss of IgE binding upon calcium depletion (Engel, Richter, Obermeyer, Briza, Kungl, Simon, Auer, Ebner, Rheinberger, Breitenbach, and Ferreira 1997; Hayek, Vangelista, Pastore, Sperr, Valent, Vrtala, Niederberger, Twardosz, Kraft, and Valenta 1998; Niederberger, Hayek, Vrtala, Laffer, Twardosz, Vangelista, Sperr, Valent, Rumpold, Kraft, Ehrenberger, Valenta, and Spitzauer 1999).

Apart from the polcalcins with two EF hands, several polcalcin-related pollen allergens with three (Bet v 3 from birch) and four EF hand domains (Ole e 8 from olive, Jun o 4 from juniper, and Amb a 10 from ragweed) have been described. IgE inhibition experiments showed that IgE reactivity to these allergens is caused by cross-reactivity of IgE originally directed to polcalcins (Tinghino et al. 2002).

3 Common Molecular Properties of Allergens

Presently, we are only beginning to understand what molecular features and characteristics may make certain proteins more allergenic than others. The level of exposure certainly plays a part. Consequently, the various sources of allergens differ between countries. Buckwheat allergy is much more common in Asia where it is widely consumed (Park, Kang, Kim, Koh, Yum, Kim, Hong, and Lee 2000). In areas where birch pollen is common, there is a higher incidence of associated apple allergy, based on the cross-reactivity of Bet v 1 with its homolog in apple, Mal d 1 (Pauli, Oster, Deviller, Heiss, Bessot, Susani, Ferreira, Kraft, and Valenta 1996). In Mediterranean countries, where birch is absent and the nsLTP from peach is one of the major allergens, atopic patients also react with the nsLTP of apples (Salcedo et al. 2004). Seeds and nuts contain highly allergenic storage proteins, which may account for 50% or more of their total protein. Most major food allergens that sensitize via the gastrointestinal tract are present in at least 1% of the total protein content of plant foods. However, some proteins that are present in all plants in very large quantities such as the enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase – 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.

3.1 Properties of Food Allergens

A compact three-dimensional structure, ligand binding, disulfide bonds, and glycosylation contribute to protein stability (Breiteneder et al. 2005c). These factors are relevant to both the resistance of proteins to denaturation by food processing and the harsh conditions of the gastrointestinal tract. Ligand binding can have the overall effect of reducing the 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 (Douliez, Jegou, Pato, Molle, Tran, and Marion 2001). nsLTPs are remarkably thermostable, retaining their allergenic activity in processed foods such as sterilized peach juice (Brenna, Pompei, Ortolani, Pravettoni, Farioli, and Pastorello 2000), beer (Asero, Mistrello, Roncarolo, Amato, and van Ree 2001), and fermented products such as wine (Garcia-Robaina, de la Torre-Morin, Sanchez-Machin, Sanchez-Monge, Barber, and Lombardero 2001). One of the structural features clearly related to stability is the presence of disulfide bonds. Both inter- and intra-chain disulfide 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 disulfide 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) (Breiteneder et al. 2005c). 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 (Pedrosa, De Felice, Trisciuzzi, and Ferreira 2000).

Several plant food allergens are able to associate with cell membranes or other types of lipid structures found in food (Breiteneder et al. 2005c). The allergenic 2S albumin from mustard was shown to interact with phospholipid vesicles (Onaderra, Monsalve, Mancheno, Villalba, Martinez del Pozo, Gavilanes, and Rodriguez 1994). This led to the proposition that such interactions might affect the uptake and processing of the allergen in the gastrointestinal tract, indicating that the biologic activity of these proteins plays a role in sustaining their allergenic potential. Similarly, there is emerging evidence that nsLTPs are also able to interact with lipid structures (Douliez, Sy, Vovelle, and Marion 2002; Subriade, Salesse, Marion, and Pézolet 1999).

The tendency of certain proteins to aggregate might affect their ability to sensitize 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 the 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 (Maleki and Hurlburt 2004; Mondoulet, Paty, Drumare, Ah-Leung, Scheinmann, Willemot, Wal, and Bernard 2005).

3.2 Properties of Pollen Allergens

There are only few publications referring to the common properties of pollen allergens. A prerequisite of a pollen protein for becoming allergenic is its rapid elution from the pollen grain after contact with the mucosa. In an examination of birch and timothy pollen, it was shown that major allergens were released from pollen grains within less than 1 min (Vrtala, Grote, Duchene, van Ree, Kraft, Scheiner, and Valenta 1993). In contrast, minor allergens such as profilins were eluted at much slower rates and heat shock protein 70, a non-allergenic protein, remained inside the pollen grain after aqueous extraction.

Allergens released from pollen grains during rainfalls frequently bind to polysaccharide granules derived from the pollen grain cytoplasm (Schäppi, Suphioglu, Taylor, and Knox 1997; Swoboda, Grote, Verdino, Keller, Singh, De Weerd, Sperr, Valent, Balic, Reichelt, Suck, Fiebig, Valenta, and Spitzauer 2004; Taylor, Flagan, Miguel, Valenta, and Glovsky 2004). Due to their small size, these particles are able to reach the lower airways and are responsible for asthma episodes in sensitized individuals after rainfalls (reviewed in D'Amato, Liccardi, D'Amato, and Holgate 2005).

Many pollen allergens families contain glycosylated allergens. Among the main families of pollen allergens, β -expansins (group 1 grass pollen allergens), Ole e 1-related allergens, polygalacturonases, berberine bridge enzymes, and pectate lyases from Cupressaceae pollen carry N-glycans. Plant glycoproteins contain N-linked glycans differing from their vertebrate counterparts by the presence of α 1,3-fucosyl and β 1,2-xylosyl residues. Therefore, these glycans are immunogenic and elicit the production of glycan-specific IgE that cross-reacts between unrelated glycoproteins from diverse plant sources. Similar to profilin-specific, cross-reactive IgE, the clinical relevance of glycan-specific IgE is usually low (reviewed in Malandain 2005). Some pollen allergens carry O-linked glycans. The major mugwort allergen, Art v 1, contains a hydroxyproline-rich C-terminal domain with a novel type of O-glycans, which bind IgE from 50% of Art v 1-sensitized patients (Leonard, Petersen, Himly, Kaar, Wopfner, Kolarich, van Ree, Ebner, Duus, Ferreira, and Altmann 2005). In contrast, a single arabinosylated hydroxyproline residue at the N-terminus of group 1 grass pollen allergens does not take part in IgE binding (Wicklein, Lindner, Moll, Kolarich, Altmann, Becker, and Petersen 2004).

Glycosylation of proteins significantly increases their hydrophilicity. It may therefore contribute to the rapid extractability of pollen allergens during

rehydration, as described above. However, glycosylation is not a requirement for pollen protein allergenicity, as members of several important families of pollen allergens such as profilins, Bet v 1-homologs, and polcalcins are not glycosylated.

In summary, most food allergens are characterized by stability to proteolytic digestion, acidic pH, and heating, while the only common feature of pollen allergens seems to be solubility and rapid extractability from the pollen grain. These different molecular requirements explain the distinct distributions of pollen and food allergens among protein families as shown in Fig. 1.

4 Sequences, Structures, and Cross-Reactivity

In general, the membership of an allergen to a protein family is calculated based on its primary sequence. Sequence identities, however, do not always indicate the degree of cross-reactivity. Cross-reactivities within the various allergen families run the whole gamut from high cross-reactivity to no cross-reactivity. Cross-reactivity is a function of antibody-accessible molecular surface. In protein families where sequence conservation and surface conservation are high, cross-reactivity will be broad. This can be observed for the profilins and polcalcins. As described above, plant profilins share at least 75% of their sequences. The distribution of conserved and variable residues on the molecular surfaces of profilins shows that the degree of surface conservation is lower than of complete sequence conservation but still high enough to explain the pronounced cross-reactivity among profilins (Radauer et al. 2006b). Similarly, sequence identities of at least 65% between polcalcins from different plant families explain their cross-reactivity (Ledesma et al. 2006; Tinghino et al. 2002).

The situation for the Bet v 1 family of proteins seems to be rather unique. The molecular surface that is seen by the antibody is much more conserved among the members of this family than their primary amino acid sequence (Jenkins, Griffiths-Jones, Shewry, Breiteneder, and Mills 2005). While Bet v 1 and its homologous allergen from apple, Mal d 1, share an amino acid sequence identity of 56%, their surfaces are 71% identical. The homolog Gly m 4 from soybean has 47% sequence identity, but a surface identity of 60% to Bet v 1. This high surface conservation explains the high degree of cross-reactivity that is the underlying cause for the oral allergy syndrome observed in many birch pollen allergic patients (Vieths et al. 2002).

A similar analysis was performed for the nsLTP family of the prolamin superfamily, comparing sequences and structures from the allergenic nsLTPs of peach (Pru p 3), corn (Zea m 14) and, wheat (Jenkins et al. 2005). The peach and corn LTPs have large proportions of their surface conserved resulting in the observed IgE cross-reactivity (Pastorello, Farioli, Pravettoni, Ispano, Scibola, Trambaioli, Giuffrida, Ansaloni, Godovac-Zimmermann, Conti, Fortunato,

and Ortolani 2000). In contrast, although some of the surface of wheat nsLTP is conserved compared to peach nsLTP, the wheat nsLTP has a very different surface topography. It seems therefore unlikely that anti-Pru p 3 IgE will cross-react with wheat nsLTP. The three-dimensional structures of the members of the family of trypsin and alpha-amylase inhibitors are more divergent than those of the nsLTPs. The wheat (Oda, Matsunaga, Fukuyama, Miyazaki, and Morimoto 1997) and the ragi (Gourinath, Alam, Srinivasan, Betzel, and Singh 2000) inhibitors are quite closely related; they share around 63% structurally aligned residues. In contrast, the wheat and the corn (Behnke, Yee, Trong, Pedersen, Stenkamp, Kim, Reeck, and Teller 1998) inhibitors share only around 30% structurally aligned residues (Mills et al. 2004). This being the case and the fact that the other type of cereal grain allergens, the cereal prolamins, show immense variety in their structures, which might explain why only a low level of cross-reactivity among cereal grains is observed (Jones, Magnolfi, Cooke, and Sampson 1995). 2S albumins do share the conserved disulfide structure common to all members of the prolamin superfamily, but they show a high level of sequence variation that is also obvious from the available structures of 2S albumins from peanut (Lehmann et al. 2006), castor bean (Pantoja-Uceda, Bruix, Gimenez-Gallego, Rico, and Santoro 2003), oil-seed rape (Rico, Bruix, Gonzalez, Monsalve, and Rodriguez 1996), and sunflower (Pantoja-Uceda, Shewry, Bruix, Tatham, Santoro, and Rico 2004). These proteins contain hypervariable loop regions that adopt a variety of conformations and are often the sites of IgE binding. This is probably the cause for the absence of IgE cross-reactivity between 2S albumins.

5 Protein Family Membership and Prediction of Allergenicity

During the past two decades, the number of identified allergens grew to several hundred. In parallel, genomics and proteomics research was extended to the organisms that represent relevant allergen sources. The development of allergen databases and associated bioinformatics tools enabled the comparison of newly identified proteins or complete proteomes or genomes to these databases (Jiang, Jasmin, Ting, and Ramachandran 2005).

Prediction of allergenicity became an important matter with the development of transgenic crops. The potential risk of introducing novel allergenic proteins into the human diet prompted the World Health Organization to develop a decision tree for the assessment of allergenicity (<http://www.fao.org/ag/agn/food/pdf/allergygm.pdf>). The algorithm assumes potential cross-reactivity if the query protein shows sequence identity to a known allergen of more than 35% over at least 80 amino acids or a contiguous stretch of at least six identical residues. However, this method produces many false-positive results; non-allergenic proteins erroneously identified as potential allergens (Stadler and Stadler 2003), especially when relying on identities of short peptides (Silvanovich, Nemeth, Song, Herman, Tagliani, and Bannon 2006). In order

to improve the performance of allergen prediction, numerous sequence or motif-based algorithms have been developed (Björklund, Soeria-Atmadja, Zorzet, Hammerling, and Gustafsson 2005; Riaz, Hor, Krishnan, Tang, and Li 2005; Saha and Raghava 2006; Stadler et al. 2003).

A review of the literature on allergenic and non-allergenic members of certain protein families reveals the limitations of all allergenicity prediction approaches. The most interesting example is the Bet v 1 family. Despite considerable cross-reactivity between family members with sequence identities below 50%, closely related isoforms from the same species can show great variability in their IgE binding capacities (Ferreira, Hirtenlehner, Jilek, Godnik-Cvar, Breiteneder, Grimm, Hoffmann-Sommergruber, Scheiner, Kraft, Breitenbach, Rheinberger, and Ebner 1996). Given the fact that non-allergenic isoforms of Bet v 1 share as much as 95% of their sequences with the major allergenic isoform, Bet v 1a, any presently available allergenicity prediction system is doomed to failure.

Some allergen families such as the polcalcins and the expansins are members of large, ubiquitous superfamilies that share common structures. However, most proteins from these superfamilies are non-allergenic. The EF hand superfamily contains several allergen families as there are polcalcins in pollen, parvalbumins as food allergens in fish and amphibians (Wild and Lehrer 2005), troponin C in cockroaches (Hindley, Wunschmann, Satinover, Woodfolk, Chew, Chapman, and Pomes 2006), and a member of the S100 family in bovine dander (Rautiainen, Rytönen, Parkkinen, Pentikainen, Linnala-Kankkunen, Virtanen, Pelkonen, and Mantylarvi 1995). However, most EF hand proteins, including ubiquitous proteins such as calmodulins, are non-allergenic. A similar situation is found in the double-psi beta barrel superfamily that contains allergenic proteins such as grass pollen β -expansins, kiwellin, and some members of the barwin family. However, α -expansins and dicot β -expansins seem to be non-allergenic just like proteins from the majority of the protein families that are members of this superfamily.

6 Concluding Remarks

During the last two decades, hundreds of allergenic proteins from all relevant sources have been identified and their sequences made available in various allergen databases. This development offered the possibility of classifying allergens into protein families. The limited distribution of allergens to few protein families confirmed the assumption that not every protein is capable of becoming an allergen. However, the elucidation of molecular features that govern the allergenicity of a protein is just in its infancy. An interdisciplinary effort involving allergology, structural biology, and bioinformatics will be required to gain new insights in this area of research.

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