Molecular Properties of Plant Food Allergens: A Current Classification into Protein Families

Michael Hauser, Matthias Egger, Michael Wallner, Nicole Wopfner, Georg Schmidt and Fatima Ferreira*

Christian Doppler Laboratory for Allergy Diagnosis and Therapy, Department of Molecular Biology, University of Salzburg, 5020 Salzburg, Austria

Abstract: So far the allergen list of the International Union of Immunological Societies (IUIS) allergen nomenclature subcommittee comprises 130 plant-derived food allergens. Based on sequence homology these allergens can be classified into only 27 out of 9,000 known protein families according to the Allfam database. These families comprise the prolamin and cupin superfamilies, pathogenesis related proteins, profilins, thaumatin-like proteins, oleosins, expansins, a number of enzymes and protease inhibitors among others. The classification on structural and thus biochemical and functional similarities will provide a new outlook on the molecules and might contribute to answer the question about allergenicity of different proteins. This will help to define clinical relevant allergenic molecules and explain cross-reactive phenomena between single food allergen sources as well as between food allergens and allergenic molecules of other origins (e.g. pollen).

Keywords: Plant food allergens, allergen protein families, properties of allergens.

INTRODUCTION

To date, the official allergen list of the International Union of Immunological Societies (IUIS) Allergen Nomenclature Subcommittee comprises 130 plant-derived food allergens (Table **1**). However, the number of genes expressed in plant tissues consumed by humans is several orders of magnitudes higher and, e.g. has been estimated within the range of 4,000 to 8,000 in the mid-endosperm development of wheat [1]. This fact raised the question about common properties rendering these few molecules capable of eliciting an allergic response in allergic individuals. Thus, in recent years several attempts to classify plant-derived food allergens by their source, clinical relevance, biologic function [2], protein fold [3], and protein families [4, 5] have been made.

According to characteristics of the clinical manifestation, plant-derived food allergens can be distinguished in class I, or complete, and class II, also termed incomplete, food allergens. In contrast to class I food allergy, that mainly affects young children with the sensitization process occurring in the gastrointestinal tract, class II allergy is predominantly seen in adults and develops as a consequence of allergic sensitization to inhalant allergens. Consequently, class II food allergens are considered of being more sensitive to heat and enzymatic digestion and therefore cannot cause per-orally sensitizations, but instead provoke allergic reactions in already sensitized patients due to IgE cross-reactivity. Depending on their stability during the digestive process these so called non-sensitizing elicitors cause symptoms ranging from mild oral inconveniencies to anaphylactic shock. They are thus responsible for pollinosis-associated food allergy, where the pollen acts as primary sensitizing agent. The most widely accepted and experimentally supported explanation for the phenomenon of pollinosis-associated food allergy is the induction of IgE directed against cross-reactive structures shared by pollen and plant-derived food. IgE cross-reactivity is determined by structural characteristics of proteins. Thus pollinosis-associated food allergies develop as a consequence of shared features at the level of primary and tertiary protein structure. It has been proposed the proteins sharing > 70% sequence identity are often cross-reactive, while those displaying < 50% rarely cross-react. However, as the cross-reactive birch and celery allergens Bet v 1 and Api g1, for example display only 40% sequence identity, this notion shall be reconsidered. In accordance, present bioinformatics supported guidelines for assessment of genetically modified crops suggest a sequence identity of 35% as cut-off for potential cross-reactivity. Exceptions may occur when post-synthetic modifications are involved in cross-reactivity between unrelated proteins. So far, several clinical pollen-food syndromes (PFS) have been described, such as the birch-fruit-vegetable-, the celery-mugwort-spice-, and the latex-fruit-syndrome, which by its molecular background is comparable with PFS [2, 6-8]. Nevertheless, a classification based on clinical symptoms does not give further insight into common molecular properties underlying allergenicity.

Indeed, most plant-derived food allergens can be integrated into only a few protein families and superfamilies on the basis of sequence homology, which is related to conserved three-dimensional structures and possibly function [1, 9]. Interestingly, many of the known plant food allergens display homology to pathogenesis-related (PR) proteins. They represent a collection of 14 unrelated protein families comprising very different plant proteins such as chitinases, glucanases, endoproteinases, peroxidases, defensins, thionins, and lipid-transfer proteins (LTPs), which by definition are induced upon environmental stress, pathogen infection, and antibiotic stimuli. Thus, for some of them antibacterial

*Address correspondence to this author at the Christian Doppler Laboratory for Allergy Diagnosis and Therapy, Department of Molecular Biology, University of Salzburg, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria; Tel: +43-6628044-5734; Fax: +43-6628044-183;
E-mail: fatima.ferreira@sbg.ac.at

1874-2262/08

and antifungal functions have been reported. Nonetheless, some of the PR-proteins are constitutively expressed in plant organs or during certain developmental stages [1, 10].

This review provides a complete list of so far known plant-derived food allergens (Table **1**) and their classification into allergen superfamilies/families (according to: www.meduniwien.ac.at/allergens/allfam and www.allergome.org). Focusing on structural characteristics leading to allergenicity, the three-dimensional structures of allergens representative for each allergen family are depicted in Fig. (**1**). In addition, allergenic members are discussed in regard to biologic function, cross-reactivity, and clinical relevance. A publication list referring to the first description of each of the 130 included allergens can be found on the IUIS Allergen Nomenclature Subcommittee homepage (www.allergen.org).

I. Prolamin Superfamily

The prolamin superfamily is termed after cereal prolamins representing major storage proteins of cereal grains. Allergens belonging to this superfamily are of low molecular weight and characterized by high contents of proline and glutamine [4]. They feature eight conserved cysteine residues and share a similar three-dimensional structure rich in α-helices [1, 11]. The prolamin superfamily comprises three major groups of plant food allergens, *i.e.* seed storage 2S albumins found in tree nuts and seeds, defense-related non-specific lipid transfer proteins (nsLTPs) found in some fruits and vegetables, and cereal α-amylase/trypsin inhibitors. In addition, a soybean hydrophobic protein Gly m 1 has been identified as an allergen and integrated into the prolamin superfamily. Due to their stability to heat and gastrointestinal digestion many allergens from this superfamily are important class I food allergens and thus, may account for severe anaphylactic reactions [12].

2S Albumins

Albumins and globulins represent the major group of seed storage proteins present in many dicotyledonous plant species [13]. They are characterized and identified on the basis of sedimentation coefficient as 2S albumins and 7S and 11S globulins [2]. 2S albumins are small globular proteins rich in arginine, glutamine, asparagine, and cysteine and comprise eight cysteine residues that are distributed in a conserved pattern (...C...C.../...CC...CXC...C...C) [14]. Due to their amino acid composition, their high content in the protein bodies of seeds and their mobilization during germination, a role as a nitrogen and sulfur donor has been proposed for these proteins. In addition, antifungal activity against a number of plant-pathogenic fungi has been shown for napins, the radish 2S albumins [15]. Typical 2S albumins, such as the napins from the *Brassicaceae* or the Brazil nut 2S albumin Ber e 1, are heterodimeric proteins consisting of two subunits of approximately 4 and 9 kDa, which are held together by conserved interchain disulfide bonds [13]. In addition to their biological interest, 2S albumins have been used by means of genetic engineering as carriers for the synthesis of biologically active peptides as well as for improving the nutritional properties of grain crops by increasing their content of essential amino acids [16].

2S albumins are an important class of common allergenic proteins present in almost all edible seeds. They originate from the *Brassicaceae* family, *i.e.* oriental mustard seed Bra j 1, rapeseed Bra n 1, turnip Bra r 1, and yellow mustard seed Sin a 1. Additionally, Brazil nut Ber e 1, black walnut Jug n 1, Jug r 1 and Jug r 4 from English walnut, Ses i 1 and Ses i 2 from sesame, Ric c 1 from castor bean, Ana o 3 from cashew nut, and Pis v 1 from pistacho have been included into the IUIS official allergen list. IgE cross-reactivity between 2S albumins from rapeseed and mustard, and members of the *Brassicaceae* family has been reported [17-19]. Due to the high frequency of systemic allergic reactions, peanut allergens deserve special attention. The glycoproteins Ara h 2, Ara h 6, and Ara h 7, which belong to the conglutin protein family, are related to the 2S albumin family of seed storage proteins. Similar to other 2S albumins, these peanut allergens are resistant to trypsin- and simulated gastric fluid proteolysis, and thus comprise an important group of class I food allergens [20].

Plant Non-Specific Lipid Transfer Proteins

Lipid transfer proteins (LTPs), originally named after their ability to transfer phospholipids between vesicles and membranes, can be divided into two types: those specific for certain classes of phospholipids and those that are able to accommodate several lipid classes, called non-specific LTPs (nsLTPs) [21]. Plant nsLTPs are closely related basic proteins, unique to flowering plants. Two subfamilies can be distinguished according to their molecular masses: the 9 kDa nsLTP1 and the 7 kDa nsLTP2 subfamily. nsLTPs are characterized by a common fold of four α-helices stabilized by four disulfide bonds forming a central hydrophobic tunnel interacting with lipophilic molecules. Nevertheless, it is now emerging that plant nsLTPs are not involved in intracellular lipid trafficking but instead, in defense against fungi and bacteria. Thus, nsLTPs have been included as member of the heterogenous collection of the PR protein families [10, 22].

nsLTPs display a wide distribution and therefore are considered as pan-allergens with sequences available from fruits, nuts, seeds, and vegetables. Thermostability as well as resistance to proteolysis and harsh pH conditions renders them potent class I food allergens [23]. They accumulate at high concentrations in the outer epidermal layers of plant organs explaining for instance the strong allergenicity of fruit peels compared to pulps as shown for *Rosaceae* fruits [24]. In fact, *Rosaceae* nsLTPs, such as apple Mal d 3, peach Pru p 3, and apricot Pru ar 3, are regarded as major allergens especially in the Mediterranean area. For example, 60 to 90% of Spanish peach-allergic individuals show positive skin prick tests to Pru p 3 [25, 26]. In contrast, the incidence of sensitization to nsLTPs in Central and Northern Europe is rather limited. A recent study demonstrated that according to *in vitro* tests only 3% of German-, but 100% of Italian patients were sensitized to the cherry nsLTP Pru av 3. However, the surprising geographical distribution pattern of sensitization to nsLTPs is still unexplained. Allergy to *Rosaceae* fruits in Central and Northern Europe seems associated with birch pollinosis and sensitization to Bet v 1-related allergens [27, 28]. It is believed, that sensitization to food nsLTPs is a consequence of food consumption and no epiphenomenon of pollen exposure. Nonetheless, there is evidence of IgE cross-reactivity between Pru p 3 and the mugwort pollen nsLTP Art v 3. Whether peach or pollen acts as primary sensitizing

Table 1. Allergens Included in the Official Allergen List

Protein Family		Botanical Family	Allergen Source		Allergen
prolamin superfamily	**2S albumins**	*Brassicales*	*Brassica juncea*	oriental mustard	Bra j 1
			Brassica napus	rapeseed	Bra n 1
			Brassica rapa	turnip	Bra r 1
			Sinapis alba	yellow mustard	Sin a 1
		Ericales	*Bertholletia excelsia*	Brazil nut	Ber e 1
		Fabales	*Arachis hypogaea*	peanut	Ara h 2
					Ara h 6
					Ara h 7
		Fagales	*Juglans nigra*	black walnut	Jug n 1
			Juglans regia	English walnut	Jug r 1
					Jug r 4
		Lamiales	*Sesamum indicum*	sesame	Ses i 1
					Ses i 2
		Malphigiales	*Ricinus communis*	castorbean	Ric c 1
		Sapindales	*Anacardium occidentale*	cashewnut	Ana o 3
			Pistacia vera	pistacho	Pis v 1
	nsLTP	*Asparagales*	*Asparagus officinalis*	asparagus	Aspa o 1
		Asterales	*Lactuca sativa*	lettuce	Lac s 1
		Brassicales	*Brassica oleracea*	cabbage	Bra o 1
		Fagales	*Castanea sativa*	chestnut	Cas s 8
			Corylus avellana	hazelnut	Cor a 8
			Juglans regia	English walnut	Jug r 3
		Poales	*Triticum aestivum*	wheat	Tri a 14
			Zea mays	maize	Zea m 14
		Rosales	*Fragaria ananassa*	strawberry	Fra a 3
			Malus domestica	apple	Mal d 3
			Prunus armeniaca	apricot	Pru ar 3
			Prunus avium	cherry	Pru a 3
			Prunus domestica	plumb	Pru d 3
			Prunus persica	peach	Pru p 3
			Pyrus communis	pear	Pyr c 3
			Rubus idaeus	raspberry	Rub i 3
		Rosids	*Vitis vinifera*	grape	Vit v 1
		Sapindales	*Citrus limon*	lemon	Cit l 3
			Citrus reticuata	tangerine	Cit r 3
			Citrus sinensis	orange	Cit s 3
		Solanales	*Lycopersicon esculentum*	tomato	Lyc e 3
	α-amylases	*Poales*	*Hordeum vulgare*	barley	Hor v 15
					Hor v 16
	cereal prolamins	*Poales*	*Hordeum vulgare*	barley	Hor v 21
			Triticum aestivum	wheat	Tri a 19
					Tri a 26
			Secale cereale	rye	Sec c 20
cupin superfamily	**germins**	*Sapindales*	*Citrus sinensis*	orange	Cit s 1
	vicillins	*Fabales*	*Arachis hypogaea*	peanut	Ara h 1
			Lens culinaris	lentil	Len c 1
			Pisum sativum	pea	Pis s 1
					Pis s 2
		Fagales	*Corylus avellana*	hazelnut	Cor a 11
			Juglans nigra	black walnut	Jug n 2
			Juglans regia	English walnut	Jug r 2
		Lamiales	*Sesamum indicum*	sesame	Ses i 3
		Sapindales	*Anacardium occidentale*	cashewnut	Ana o 1
			Pistacia vera	pistacho	Pis v 3

(Table 1) contd…..

Protein Family		**Botanical Family**	**Allergen Source**		**Allergen**
cupin superfamily	**legumins**	*Fabales*	*Arachis hypogaea*	peanut	Ara h 3
					Ara h 4
			Bertholletia excelsia	Brazil nut	Ber e 2
		Fagales	*Corylus avellana*	hazelnut	Cor a 9
		Lamiales	*Sesamum indicum*	sesame	Ses i 6
					Ses i 7
		Sapindales	*Anacardium occidentale*	cahsewnut	Ana o 2
			Pistacia vera	pistacho	Pis v 2
PR proteins	**PR-1**	*Cucurbitales*	*Cucumis melo*	muskmelon	Cuc m 3
	PR-3 (class I chitinases)	*Brassicales*	*Brassica rapa*	turnip	Bra r 2
		Fagales	*Castanea sativa*	chestnut	Cas s 5
		Laurales	*Persea americana*	avocado	Pers a 1
		Poales	*Triticum aestivum*	wheat	Tri a 18
	PR-5 (TLPs)	*Ericales*	*Actinidia deliciosa*	kiwi	Act d 2
		Rosales	*Malus domestica*	apple	Mal d 2
			Prunus avium	cherry	Pru av 2
		Solanales	*Capsicum annuum*	bell pepper	Cap a 1
	PR-10 (Bet v 1-related)	*Apiales*	*Apium graveolens*	celery	Api g 1
			Daucus carota	carrot	Dau c 1
		Ericales	*Actinidia chinensis*	gold kiwi	Act c 6
		Fabales	*Arachis hypogaea*	penaut	Ara h 8
			Glycine max	soybean	Gly m 4
			Vigna radiata	mungbean	Vig r 1
		Fagales	*Corylus avellana*	hazelnut	Cor a 1
		Rosales	*Fragaria ananassa*	strawberry	Fra a 1
			Malus domestica	apple	Mal d 1
			Prunus armeniaca	apricot	Pru ar 1
			Prunus avium	cherry	Pru av 1
			Prunus persica	peach	Pru p 1
			Pyrus communis	pear	Pyr c 1
			Rubus idaeus	raspberry	Rub i 1
enzymes and protease inhibitors	**thioredoxins**	*Poales*	*Triticum aestivum*	wheat	Tri a 25
			Zea mays	maize	Zea m 25
	isoflavone reductases	*Rosales*	*Pyrus comunis*	pear	Pyr c 5
	β-amylases	*Poales*	*Hordeum vulgare*	barley	Hor v 17
	glycoside hydrolases	*Rosales*	*Ziziphus mauritiana*	Chinese date	Ziz m 1
		Solanales	*Lycopersicon esculentum*	tomato	Lyc e 2
	patatin family	*Solanales*	*Solaneum tuberosum*	potato	Sola t 1
	papain-like cysteine proteases	*Bromeliales*	*Ananas comosus*	pineapple	Ana c 2
		Ericales	*Actinidia deliciosa*	kiwi	Act d 1
		Fabales	*Glycine max*	soybean	Gly m 1
	subtilisin-like serine proteases	*Cucurbitales*	*Cucumis melo*	muskmelon	Cuc m 1
	berberine bridge enzymes	*Apiales*	*Apium graveolens*	celery	Api g 5
	cystatins	*Ericales*	*Actinida deliciosa*	kiwi	Act d 4
	kunitz-type protease inhibitors	*Solanales*	*Solaneum tuberosum*	potato	Sola t 2
					Sola t 3
					Sola t 4
others	**profilins**	*Apiales*	*Apium graveoles*	celery	Api g 4
			Daucus carota	carrot	Dau c 4
		Bromeliales	*Ananas comosus*	pineapple	Ana c 1
		Cucurbitales	*Cucumis melo*	muskmelon	Cuc m 2
		Fabales	*Arachis hypogaea*	peanut	Ara h 5
			Glycine max	soybean	Gly m 3
		Fagales	*Corylus avellana*	hazelnut	Cor a 2
		Poales	*Hordeum vulgare*	barley	Hor v 12
			Oryza sativa	rice	Ori s 12

(Table 1) contd…..

Protein Family		Botanical Family	Allergen Source		Allergen
others	**profilins**	*Rosales*	*Fragaria ananassa*	strawberry	Fra a 4
			Malus domestica	apple	Mal d 4
			Prunus avium	cherry	Pru a 4
			Prunus dulcis	almond	Pru du 4
			Prunus persica	peach	Pru p 4
			Pyrus communis	pear	Pyr c 4
		Sapindales	*Citrus sinensis*	orange	Cit s 2
			Litchi chinensis	litchi	Lit c 1
		Solanales	*Capsicum annuum*	bell pepper	Cap a 2
			Lycopersicon esculentum	tomato	Lyc e 1
		Zingiberales	*Musa x paradisiaca*	banana	Mus xp 1
	oleosins	*Lamiales*	*Sesamum indicum*	sesame	Ses i 4
					Ses i 5
	expansins	*Ericales*	*Actinidia deliciosa*	kiwi	Act d 5
		Fabales	*Glycine max*	soybean	Gly m 2
	chlorophyll-binding proteins	*Apiales*	*Apium graveolens*	celery	Api g 3
	seed specific biotinylated protein	*Fabales*	*Lens culinaris*	lentil	Len c 2
	60S acidic ribosomal binding protein	*Rosale*	*Prunus dulcis*	almond	Pru du 5
	unidentified	*Ericales*	*Actinida deliciosa*	kiwi	Act d 3
		Sapindales	*Pistacia vera*	pistacho	Pis v 4

source is still a matter of debate [29, 30]. Other nsLTPs originating from the botanical family of *Rosaceae* comprise strawberry Fra a 3, plum Pru d 3, pear Pyr c 3, and raspberry Rub i 3.

IgE cross-reactivity requires sequence homology that was found in various degrees (from 30% to 95%) between members of the LTP family from different species [31]. For example, walnut Jug r 3, cabbage Bra o 3, and maize Zea m 14 nsLTPs have been demonstrated to cross-react with peach Pru p 3. Grape Vit v 1 was characterized and included as an elicitor of the proposed "LTP-associated-clinical-syndrome" explaining allergic cross-reactions to nsLTPs originating from multiple allergen sources [32].

In addition, lettuce Lac s 1, tomato Lyc e 3, asparagus Aspa o 1, and wheat Tri a 14 have been identified as allergenic nsLTPs. Cor a 8 has been demonstrated to be a clinically important hazelnut allergen causing severe allergic reactions in Spanish patients. Recently, nsLTPs originating from chestnut (Cas s 8) and citrus fruits, *i.e.* Cit l 3 from lemon, Cit r 3 from tangerine, and Cit s 3 from sweet orange have been included into the official IUIS allergen list.

α-Amylases

The α-amylase family or family13 hydrolases comprises a number of starch-converting enzymes sharing common characteristics such as an eight-stranded α/β barrel structure, the ability to hydrolyze 1,4-α-D-glucosidic linkages of attached polysaccharides in α-conformation, and conserved amino acid residues in the enzyme's active sites [33].

To date, several α-amylases have been identified in diverse organisms e.g. Aed a 4 in yellow fever mosquito or Der p 4 in European house dust mite. Cereal α-amylases (e.g. barley Hor v 15 and Hor v 16) are important allergens for patients with baker's asthma. Some of these patients also display IgE reactivity to fungal α-amylases, e.g. Asp o 21 from *Aspergillus*, used as baking additives or present in mold-contaminated flour.

Cereal Prolamins

Cereal prolamins are major storage proteins of the cereal grain endosperm and are named glutenins and gliadins in wheat, secalins in rye, and hordeins in barley. These sulfur rich proteins are composed of an N-terminal domain of proline- and glutamin- rich repeats and a C-terminal domain responsible for intrachain disulfide bonds [1]. So far, γ-3 hordein (Hor v 21) from barley, Sec c 20 from rye, as well as Tri a 19 and Tri a 26 from wheat are included in the IUIS allergen list.

II. Cupin Superfamily

Cupins are named after their conserved β-barrel fold ('*cupa*' is the Latin term for small barrel) and comprise a large superfamily of proteins sharing a common origin. Their evolution can be followed from bacteria to eukaryotes including animals and higher pants. Two functional classes can be divided, *i.e.* the monocupins and bicupins, containing one or two conserved cupin domains, respectively [34].

Germins

Germins, bacterial carbohydrate isomerases and epimerases, as well as germin-like proteins represent the monocupins. However, so far orange Cit s 1 is the only member of this family that has been reported to act as an allergen.

Vicillins

Mature 7/8S globulins (vicillins or 7S vicillin-like globulins) are homotrimeric proteins of about 150 to 190 kDa lacking cysteines and therefore disulfide bonds. Their detailed subunit compositions vary considerably due to differences in proteolytic processing and glycosylation of the monomers.

Ara h 1, a major peanut allergen, represents the best-characterized allergenic vicillin. Further allergens belonging to this protein family include Jug r 2 from English walnut, Jug n 2 from black walnut, Cor a 11 from hazelnut, Ana o 1 from cashew nut, Ses i 3 from sesame, Pis s 1 and Pis s 2 from pea, Pis v 3 from pistacho, and Len c 1 from lentil [1, 34].

Legumins

Mature 11S globulins are hexameric proteins composed of two trimers that are proteolytically processed to yield an acidic 30-40 kDa polypeptide linked by a disulfide bond to a basic polypeptide of about 20 kDa.

Allergenic legumins include the minor peanut allergens Ara h 3 and Ara h 4, previously described as distinct allergens with high sequence similarity to glycinins but now considered to be the same allergens. Other described legumins are Cor a 9 from hazelnut, Ber e 2 from Brazil nut, Pis v 2 from pistacho, Ana o 2 from cashew nut, soybean glycinin, and sesame Ses i 6 and Ses i 7 [1, 34].

III. Pathogenesis-Related Proteins

PR-proteins constitute a collection of 14 unrelated protein families, which by definition are induced upon environmental stress, pathogen infection, and antibiotic stimuli. Interestingly, many plant-derived food allergens can be found within this group of proteins, *i.e.* in the PR-families 1, 3, 5, 10, and 14 (the latter are nsLTPs belonging to the prolamin superfamily). In general, PR-proteins are rather small in size, stable at low pH, and resistant to proteolysis, making them good candidates for provoking an immune response in predisposed individuals.

PR-1 Family

The muskmelon allergen Cuc m 3 is the only plant food allergen within the PR-1 family, which comprises many pollen allergens, thus delivering the first evidence of the involvement of this plant allergen family in food allergy [35].

PR-3 Family or Class I Chitinases

In general, chitinases catalyze the hydrolysis of β-1,4-N-acetyl-D-glucosamine linkages in chitin polymers, thus playing a role in plant defense against fungal and insect pathogens [36]. Class IA/I and IB/II enzymes differ in the presence (IA/I) or absence (IB/II) of an N-terminal hevein-like chitin-binding domain. This domain is thought to be involved in recognition or binding of chitin subunits.

Due to the presence of the hevein-like domain, class I chitinases, e.g. latex Hev b 11 [37], and plant food allergens such as avocado Pers a 1 and chestnut Cas s 5 cross-react with the major latex allergen hevein [38, 39]. Further, there are some evidences that class I chitinases from cherimoya, passion fruit, kiwi, papaya, mango, and tomato display IgE cross-reactivity with hevein [40], although the catalytic domains of these allergens showed comparably low IgE binding activities [41]. As the hevein domain comprises most of the molecule's allergenicity, two more allergens should be mentioned although displaying no chitinase activity, *i.e.* Bra r 2 (turnip rape) with a prohevein-like domain composition [42] and the wheat germ agglutinin Tri a 18, a lectin, consisting of four hevein-like domains. In summary, most of the allergenic activity of chitinases seems to be due to the hevein-like domain. Due to their potential high cross-reactivity, these allergens have been implicated in the latex-fruit-syndrome.

PR-5 Family or Thaumatin-Like Proteins

The family of thaumatin-like proteins (TLPs) can be classified into three groups: (i) those produced in response to pathogen infection, (ii) produced in response to osmotic stress (osmotins), and (iii) antifungal proteins present in cereals [2]. The majority of TLPs has a molecular mass of about 20kDa, consists mainly of anti-parallel β-sheets, and is stabilized by eight disulfide bonds. TLPs are generally resistant to proteolytic degradation and pH- or heat-induced denaturation [43].

Among other allergen sources, allergenic TLPs can be found in several fruits such as apple Mal d 2, cherry Pru av 2, bell pepper Cap a 1, and kiwi Act d 2, as well as in orange and grape.

PR-10 Family or Bet v 1-Related Proteins

The major birch pollen allergen Bet v 1 and its homologues from other pollen as well as food sources belong to the PR-10 protein family. In general, the expression of these proteins in plants can be stress-induced, though high levels of these PR-10 proteins are also found in reproductive tissue such as pollen, fruits, or seeds. Despite a rather low amino acid sequence similarity of Bet v 1 and its allergenic homologues, a comparison of three-dimensional structures of Bet v 1, Api g 1 from celery, and Pru av 1 from peach revealed a much conserved folding pattern of the allergens [44-46], which for sure affects the high level of patients´ IgE cross-reactivity towards the Bet v 1 family members. Structural protein analysis revealed a common α-β fold with a solvent-accessible cavity traversing the protein. In addition, the binding capacity of this cavity for deoxycholate that is similar to brassinosteroids (ubiquitous plant steroid hormones) indicates a possible role for Bet v 1 as plant steroid carrier [47]. This fact is supported by recent data of Mogensen *et al.* [48] demonstrating that the protein can bind to membranes, which might contribute to its role of transferring ligands between cells or tissues. In contrast, other studies demonstrated a much broader binding specificity of PR-10 proteins towards a variety of biological ligands including fatty acids, flavonoids, and cytokinins as well as ribonuclease activities. Nevertheless, the biological function of Bet v 1 is not yet fully understood [49].

As Bet v 1-related proteins are relatively heat-labile and unstable towards digestion they are generally considered as class II food allergies with Bet v 1 acting as sensitizing agent and leading to the induction of cross-reactive IgE antibodies in atopic individuals. The symptoms are usually moderate and mainly affecting the oropharynx. However, severe forms of PFS including anaphylaxis have been attributed to the Bet v 1 homologue Gly m 4 from soybean [1, 12].

Among the foods most commonly implicated with Bet v 1-associated allergies are fruits of the *Rosaceae* (e.g. apple

Mal d 1, pear Pyr c 1, peach Pru p 1, cherry Pru av 1, apricot Pru ar 1, and strawberry Fra a 1) or *Fabaceae* family (peanut Ara h 8, mung bean Vig r 1), but also nuts (hazelnut Cor a 1) and *Apiaceae* vegetables (celery Api g 1, carrot Dau c 1) display high levels of cross-reactivity towards the major birch pollen allergen. Additionally, gold kiwi Act c 6 has been recently identified as allergen and included in the official IUIS allergen list.

IV. Enzymes and Protease Inhibitors

The following chapter reviews plant food allergens that act as enzymes (reductases, amylases, hydrolases, transferases, and proteases) or protease inhibitors and cannot be classified within the already mentioned superfamilies. It has been suggested that biochemical or enzymatic activities of allergens may contribute to their sensitizing potential. This idea is supported by the observation that the cysteine-protease activity of the major dust mite allergen Der p 1 has as pro-allergenic adjuvant effect. It increases IgE production by cleaving the low affinity IgE receptor on B cells and monocytes and decreases Th1 cell proliferation by cleaving the IL-2 receptor. Furthermore, by disrupting tight junctions it enhances permeability in the bronchial epithelium [50, 51]. However, it is unclear if enzymatic function in general influences allergenicity. In fact, most allergens do not posses enzymatic activity.

Thioredoxins

Thioredoxins are small ubiquitous enzymes present in many species from *Archaebacteria* to man. They serve as general protein disulfide oxido-reductases interacting with a broad range of proteins. Allergenic thioredoxins have been identified in fungi as well as in plants. The thioredoxins from wheat (Tri a 25) and maize (Zea m 25) are related to baker's asthma, an occupational disease affecting 4% to 10% of bakery workers in European countries [52].

Isoflavone Reductases

Isovlavone reductases (IFR) belong to the nicotinamide adenine dinucleotide phosphate (NADP)-dependent oxidoreductases that are involved in plant secondary metabolism [53]. Allergens from this family include the minor birch pollen allergen Bet v 6, which has been demonstrated to cross-react with IFR from pear (Pyr c 5), lichee, and sharon fruit [54, 55]. Additionally, Bet v 6-specific serum IgE recognized bands at the corresponding size in apple mango, banana, and carrot extracts [56]. There are also reports on immunoreactive IFRs of orange and latex. Although these allergens are not considered as major allergens, IFRs can be classified as panallergens with members in pollen, fruits, and latex.

β-Amylases

β-amylases are enzymes belonging to the group of exoamylases, which hydrolyze 1,4-α glucosidic linkages in starch-type polysaccharide substrates. These enzymes are found in a large variety of microorganisms as well as in plants where they can provoke allergic reactions as demonstrated for the barley allergen Hor v 17 [33, 57].

Glycoside Hydrolases

This widespread group of enzymes hydrolyzes glycosidic bonds and has been classified into 85 subfamilies. The glycoprotein Lyc e 2, a fructofuranosidase from tomato, belongs to the glycoside hydrolase family 32 and represents a major allergen of tomato recognized by more than 50% of tomato-allergic individuals. However, one third of the sera from tomato-allergic patients show glycan specific antibodies leading to the speculation that the glycans of Lyc e 2 might contribute to its IgE binding properties [58]. The protein is highly concentrated in the red ripening stage of the fruit. If ripening is inhibited like in the ripening inhibitor mutant tomato, the accumulation of β-fructofuranosidase and consequently the serum IgE reactivity to the extract is reduced [59]. So far, there are no reports of β-fructofuranosidases of other species that elicit allergy, though serum IgE cross-reactivity to molecules with similar carbohydrate moieties cannot be excluded.

Further, Ziz m 1 from Chinese date belonging to the glycoside hydrolase sub-family 18 has been recently identified as allergen [60].

Patatin Family

The patatin family consists of various glycoproteins in plants making up more than 40% of the total soluble protein in potato tubers. Patatins serve as storage proteins and it has been demonstrated that they exhibit both lipid acyl hydrolase and acetyl transferase activities, which might be involved in tissue wounding responses. Further, recent studies report on antioxidant activities of the major potato allergen Sola t 1 [61].

Moreover, potato patatin is homologous and cross-reacts with the major latex allergen Hev b 7. This could be one explanation for the association of potatoes with the latex-fruit-syndrome.

Papain-Like Cysteine Proteases

Members of the papain family are widespread in nature and are cysteine proteases synthesized as inactive proenzymes with N-terminal pro-peptide regions. The pro-peptide plays an important role as inhibitor of enzymatic activity and for the correct folding of the newly synthesized protein. Mature enzymes are generally 25-28 kDa proteins [62]. So far, a number of allergens belonging to the papain family have been identified including inhalant allergens such as the major dust mite allergen Der p 1 and also plant food allergens such as Ana c 2 bromelain from pineapple, Act d 1 actinidin, the major allergen from kiwi fruit, and Car p 1 papain from papaya [63]. Gly m 1, a major allergen from soybean seed storage vacuoles shows sequence similarities to papain-like proteases but lacks enzymatic activity.

Subtilisin-Like Serine Proteases

Subtilases comprise the second largest family of serine proteases characterized to date with members in *Archaebacteria*, eukaryotes, and even viruses. Surprisingly, the catalytic domains of subtilases display a high degree of sequence variability with exception of three catalytic residues (Asp-His-Ser). Apart from that, the structures show no further similarities [64].

Most allergens from this family are fungal allergens belonging to the subfamilies of alkaline or vacuolar serine proteases [65]. So far, the only plant food allergen belonging to this family is Cuc m 1 (cucumisin) from muskmelon. Although homologues of cucumisin were reported in other plants like soybean, tomato, latex, rice, barley, *etc*. allergenicity and cross-reactivity require further investigation.

Berberine-Bridge Enzymes

Berberine bridge and berberine bridge-like enzymes are involved in the biosynthesis of numerous isoquinoline alkaloids. These flavoproteins catalyze the transformation of the N-methyl group of (S)-reticuline into the C-8 berberine bridge carbon of (S)-scoulerine [66]. The first allergen belonging to this family was found in bermuda grass pollen. Phl p 4 from timothy grass also shows homology to berberin-bridge enzymes, similar as Api g 5 from celery tuber, and a high molecular weight allergen from *Brassica napus* pollen. All these allergens are glycoproteins and seem to bind human IgE if not exclusively, then prominently *via* their N-liked glycan moieties. For example, recombinant Phl p 4 produced in *Escherichia coli* displays a significantly lower IgE binding capacity compared to the natural, glycosylated allergen [67]. Api g 5, the so far only known food allergen within this group, has been suggested to be involved in the “celery-mugwort-spice-syndrome”. Carbohydrate analysis of the allergen revealed the presence of glycans carrying fucosyl and xylosyl residues, structures previously shown to bind IgE [68-70].

Cystatins

Cystatins belong to the cystatin superfamily of reversibly binding cysteine protease inhibitors, which can be subdivided into three animal and one plant cystatin family [71]. Most phytocystatins are 12-14 kDa in size, contain no disulfide bonds, and show significant amino acid sequence similarity to the cystatin families of animal origin [72]. Plant seed cystatins are now understood as factors controlling germination by inhibition of endogenous cysteine proteinases [73]. Oryzacystatin from rice seeds was identified as the first well-defined cystatin of plant origin [74]. Although many cloning approaches led to the identification of various plant cytstatins including those of strawberry, sunflower, wheat, barley, corn, and soybean, so far allergenic phytocystatins were found only in kiwifruit (Act d 4), golden kiwi, and in the pollen of short ragweed (*Ambrosia artemisiifolia*) [75].

Kunitz-Type Protease Inhibitors

Protease inhibitors displaying a Kunitz-type domain protect plants against insect attacks, inhibiting enzymes of the digestive system, like trypsin. Regarding allergenicity, the most prominent member of this group is the soybean trypsin inhibitor. Although only 20% of soybean-allergic patients react with this soybean allergen, food induced anaphylactic shocks have been reported [76, 77]. In addition to its presence in soy, the soybean trypsin inhibitor is also found as a contaminant in lecithin [78], which is widely used as emulgating agent and represents a potential risk for allergic patients. Furthermore, the soybean trypsin inhibitor is considered highly resistant to thermal and chemical denaturation [79]. So far, only the potato Kunitz-type protease-inhibitors Sola t 2 (a cathepsin inhibitor), Sola t 3 (a cystein protease inihibtor), and Sola t 4 (a serine protease inhibitor) have been included in the official IUIS allergen list. As in enzyme-linked immunosorbent assays 51% of atopic children showed specific IgE to Sola t 2, 43% to 58% to different isoforms of Sola t 3, and 67% to Sola t 4, respectively, they represent major allergens in early life.

V. Others

Profilins

Profilins represent an allergen family of small 12 to 15 kDa cytosolic actin binding molecules and belong to the α-β class of proteins featuring mainly anti-parallel β–sheets. They play a key role in cell motility through the regulation of actin microfilament polymerization dynamics. In plant cells they are thus involved in processes like cytokinesis, cytoplasmatic steaming, cell elongation, and the growth of pollen tubes and root hairs [80, 81]. However, being involved in essential cellular processes, profilins can be found in all organisms examined so far, therefore being considered as pan-allergens, which are responsible for many cross-reactions between inhalant and nutritive allergen sources [82, 83]. In higher plants profilins constitute a family of highly conserved proteins displaying sequence identities of at least 75%, even between members from distantly related organisms. However, as sequence conservation is reflected by highly similar three-dimensional structures, IgE cross-reactivity between profilins seems to be a result of the highly conserved three-dimensional profilin fold [84].

It has been reported that about 20 to 36% of all pollen-sensitized patients recognize profilin [82, 85]. Since profilin specific IgE cross-reacts with homologues from virtually every plant source, sensitization to these allergens is a risk factor for allergic reactions to multiple pollen sources and for the development of class II food allergy [86]. As described for other class II food allergens, profilins are sensitive to heat denaturation and gastric digestion leading to allergic reactions that are usually confined as moderate oral symptoms elicited by raw food [1, 87]. In contrast, celery profilin Api g 4 is known to be partially heat resistant, and might therefore also elicit allergic reactions after cooking [88, 89].

To date, several plant food-derived profilins have been characterized, most of them being evidently involved in PFS. Hazelnut profilin Cor a 2 and the profilins from *Rosaceae* fruits strawberry Fra a 4, apple Mal d 4, cherry Pru av 4, almond Pru du 4, peach Pru p 4, and pear Pyr c 4 for example, are considered to cross-react with grass and/or birch pollen Bet v 2 [90], the first identified plant profilin [91]. Celery Api g 4 and carrot Dau c 4 play a role in the celery-mugwort-spice syndrome, whereas melon Cuc m 2 and banana Mus xp 1 are involved in the ragweed-melon-banana association [6]. Furthermore, profilin is considered an important mediator in IgE cross-reactivity between pollen and exotic fruit, like lychee Lit c 1 [92, 93] and pineapple Ana c 1. In addition, IgE binding profilins from peanut Ara h 5, soybean Gly m 3, orange Cit s 2, bell pepper Cap a 2, and tomato Lyc e 1 were produced as recombinant proteins. Finally, two profilins originating from the botanical family of *Poaceae*, *i.e.* barley Hor v 12 and rice Ory s 12, have been identified and included into the official IUIS allergen list.

Oleosins

Oleosins are hydrophobic plant proteins found only in association with small storage oil drops. These oil bodies are discrete spherical organelles, mainly composed of triacylglycerols and are surrounded by a phospholipids/oleosin annulus. Several oleosins were lately described, confirming

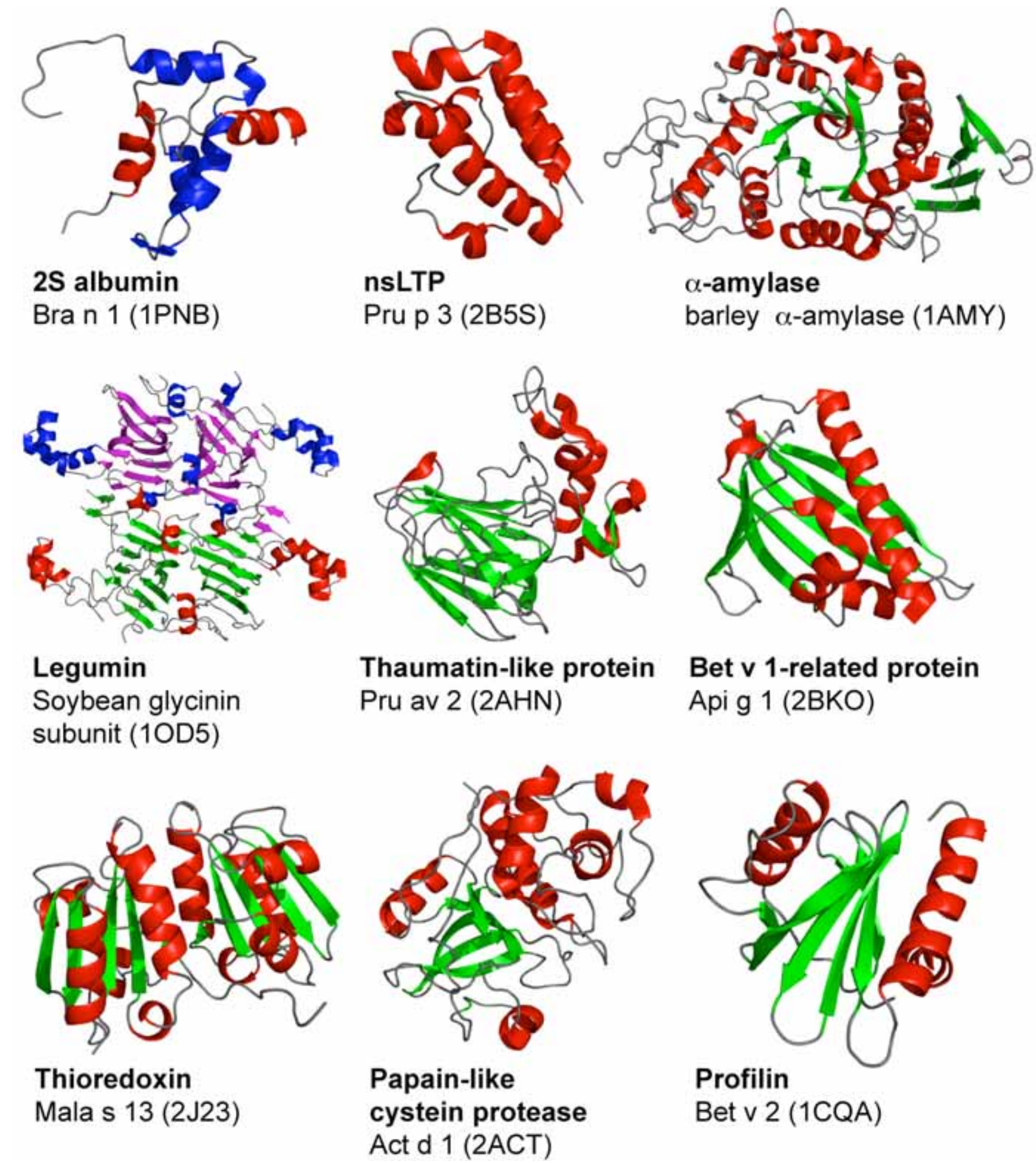

Fig. (1). Illustration of the three-dimensional structures of allergens, each representing a distinct protein family. For single chain proteins alpha-helical structures are presented in red, beta-sheets in green. In additional chains of oligomeric allergens alpha-helices and beta-sheets are depicted in blue and magenta, respectively. The PDB (www.pdb.org) codes are given in parantheesis.

that all of them comprise three distinct domains: a conserved hydrophobic domain of about 70 amino acid residues being particularly rich in aliphatic amino acids flanked by an N- and a C-terminal domain, which are more hydrophilic with less conserved amino acid sequences. Allergenic oleosins were identified in sesame (Ses i 4 and Ses i 5), nuts (peanut and hazelnut oleosins), legumes, and seeds [94, 95].

Expansins

Kiwellin (Act d 5) is a 28kD allergen from kiwi fruits (*Actinidia chinensis*) related to ripening-related proteins from grape, potato, and rice. Comparative analysis of the hypothetical tertiary structure of kiwellin showed fold similarities with the major grass pollen allergen Phl p 1, a cysteine-rich glycoprotein representing one of the most important aeroallergens known to date. Similarities were also found with Barwin-like proteins sharing a structural motif with various wound-induced and other pathogenesis-related proteins from potato, rubber trees, and tobacco [96].

Chlorophyll-Binding Proteins

Api g 3, which was isolated from a celery cDNA library with celery-allergic patients´ sera, was identified as chlorophyll A/B binding protein that together with chlorophylls A and B constitutes the light-harvesting complex of photosynthetic organisms. This complex functions as light receptor capturing and delivering excitation energy to plant photosystems I and II. However, studies on the immunological characterization of Api g 3 are still lacking [97].

CONCLUDING REMARKS

Presently, around 9,000 different protein families are defined within the protein database Pfam (http://www.sanger.ac.uk/Software/Pfam/which), which covers most of the known protein sequences. The 130 plant-derived food allergens included in the official IUIS allergen list can be assigned to only 27 of these protein families, though the growing knowledge about allergenic molecules will eventually lead to the discovery of new allergens belonging to additional protein families. The recently identified pistacho allergen Pis v 4 for example seems to be a manganese superoxide dismutase. In addition, lentil Len c 2 (identified as seed-specific biotinylated protein), almond Pru du 5 (a 60S acidic ribosomal protein), and a novel kiwi allergen termed Act d 3 have been included in the official IUIS allergen list. However, Act d 3 could not be assigned to a plant food allergen family.

In general, any innocuous environmental antigen that is able to trigger a Th2 or an IgE response is defined as allergen. Depending on the percentage of allergic individuals reacting to a protein of a given allergenic source, major (> 50%) and minor (< 50%) allergens can be distinguished. Both major and minor allergens can be found in every plant food protein family. The seed storage proteins for example are considered as major allergens of the prolamin superfamily that are likely to act as potent class I food allergens. In contrast, profilins and PR-proteins are regarded as class II food allergens due to their instability to heat and gastric digestion. For instance, profilin has been identified as a major allergen in patients suffering from pollen-food syndrome caused by melon. Digestibility analysis of melon profilin revealed its stability in human saliva but not in simulated gastric fluid, which is typical for class II food allergens [87].

Individuals suffering from the birch-fruit-vegetable display mild reactions upon ingestion of raw birch-associated foods but tolerate these foods after heating or cooking. This is due to susceptibility of the Bet v 1-food homologues to heat and gastric digestion, which leads to denaturation or destruction of conformational-dependent IgE epitopes. However, birch pollen-related foods have been shown to be effective activators of T cells specific for homologous allergens in pollen source [98], demonstrating that T cell epitopes are not affected by heat or gastric digestion and can contribute to worsening of the disease.

The clinical relevance and manifestations in class I and class II food allergy, as well as classification as major and minor allergens are not always obvious and are subject to geographical discrepancies. LTPs for example are thermostable and extremely resistant to pepsin digestion and thus are likely to act as potent class I food allergens. Indeed, they act as major allergens in the Mediterranean area where they are considered as the most important allergens of *Rosaceae* fruits. This is illustrated by peach LTP, Pru p 3, which is considered the major class I allergen for the Spanish population. However, in other geographical areas (e.g. Central Europe) where allergy to peach is rather a consequence of sensitization to birch pollen Bet v 1, peach Pru p 3 is not a major allergen. The situation is becoming increasingly complicated by recent findings [29] showing that mugwort pollen LTP behaves as the primary sensitizing allergen in patients with IgE to both mugwort and peach LTP, rendering Pru p 3 a class II food allergen. Thus, the idea that allergic reactions caused by cross-reactivity between a sensitizer and an elicitor raises the question of which antigens should be called allergens: the sensitizers, the symptom elicitors, or both?

The classification of allergens into protein families provides an overview on allergen distribution and allows insights into the clinical relevance in terms of cross-reactive allergy syndromes that may worsen the prognosis of allergy. In addition, it might contribute to answer the question of allergenicity of different proteins, help to define clinically relevant allergenic molecules, and to explain the cross-reactivity phenomena between food allergen sources as well as food allergens and allergenic molecules of other origins (e.g. pollen). So far, attempts to identify common properties of allergens that would render them allergenic have failed. Aspects of protein structure that are likely relevant for allergenicity are solubility, stability, size, and compactness of the overall fold. In fact, most allergens are relatively small, hydrophilic, and stable proteins, apparently lacking bacterial homologues [99]. These aspects reflect the dependency of allergenicity on transport over mucosal barriers and susceptibility to proteases. The findings that most allergens identified so far belong to a limited set of protein families does not support the assumption that in principle every protein could be an allergen. Nonetheless, the burning question what makes a protein an allergen still remains unanswered.

ACKNOWLEDGEMENTS

The work of the authors was supported by grants from the Christian Doppler Research Association and the Oesterreichische Nationalbank (Project Nr. 12533).

REFERENCES

[1] Breiteneder H, Radauer C. A classification of plant food allergens. J Allergy Clin Immunol 2004; 113(5): 821-30; quiz 31.

[2] Breiteneder H, Ebner C. Molecular and biochemical classification of plant-derived food allergens. J Allergy Clin Immunol 2000; 106(1 Pt 1): 27-36.

[3] Aalberse RC. Structural biology of allergens. J Allergy Clin Immunol 2000; 106(2): 228-38.

[4] Shewry PR, Beaudoin F, Jenkins J, Griffiths-Jones S, Mills EN. Plant protein families and their relationships to food allergy. Biochem Soc Trans 2002; 30(Pt 6): 906-10.

[5] Breiteneder H, Ebner C. Atopic allergens of plant foods. Curr Opin Allergy Clin Immunol 2001; 1(3): 261-7.

[6] Egger M, Mutschlechner S, Wopfner N, Gadermaier G, Briza P, Ferreira F. Pollen-food syndromes associated with weed pollinosis: an update from the molecular point of view. Allergy 2006; 61(4): 461-76.

[7] Sicherer SH. Clinical implications of cross-reactive food allergens. J Allergy Clin Immunol 2001; 108(6): 881-90.

[8] Yagami T. Allergies to cross-reactive plant proteins. Latex-fruit syndrome is comparable with pollen-food allergy syndrome. Int Arch Allergy Immunol 2002; 128(4): 271-9.

[9] Breiteneder H, Clare Mills EN. Plant food allergens--structural and functional aspects of allergenicity. Biotechnol Adv 2005; 23(6): 395-9.

[10] Hoffmann-Sommergruber K. Pathogenesis-related (PR)-proteins identified as allergens. Biochem Soc Trans 2002; 30(Pt 6): 930-5.

[11] Kreis M, Forde BG, Rahman S, Miflin BJ, Shewry PR. Molecular evolution of the seed storage proteins of barley, rye and wheat. J Mol Biol 1985; 183(3): 499-502.

[12] Breiteneder H, Mills EN. Molecular properties of food allergens. J Allergy Clin Immunol 2005; 115(1): 14-23, quiz 4.

[13] Shewry PR, Napier JA, Tatham AS. Seed storage proteins: structures and biosynthesis. Plant Cell 1995; 7(7): 945-56.

[14] Pantoja-Uceda D, Bruix M, Santoro J, Rico M, Monsalve R, Villalba M. Solution structure of allergenic 2 S albumins. Biochem Soc Trans 2002; 30(Pt 6): 919-24.

[15] Terras FR, Schoofs HM, De Bolle MF, *et al*. Analysis of two novel classes of plant antifungal proteins from radish (Raphanus sativus L.) seeds. J Biol Chem 1992; 267(22): 15301-9.

[16] Altenbach SB, Kuo CC, Staraci LC, *et al*. Accumulation of a Brazil nut albumin in seeds of transgenic canola results in enhanced levels of seed protein methionine. Plant Mol Biol 1992; 18(2): 235-45.

[17] Monsalve RI, Gonzalez de la Pena MA, Lopez-Otin C, *et al*. Detection, isolation and complete amino acid sequence of an aeroallergenic protein from rapeseed flour. Clin Exp Allergy 1997; 27(7): 833-41.

[18] Pastorello EA, Farioli L, Pravettoni V, *et al*. Sensitization to the major allergen of Brazil nut is correlated with the clinical expression of allergy. J Allergy Clin Immunol 1998; 102(6 Pt 1): 1021-7.

[19] Pastorello EA, Pompei C, Pravettoni V, *et al*. Lipid transfer proteins and 2S albumins as allergens. Allergy 2001; 56(Suppl 67): 45-7.

[20] Murtagh GJ, Dumoulin M, Archer DB, Alcocer MJ. Stability of recombinant 2 S albumin allergens *in vitro*. Biochem Soc Trans 2002; 30(Pt 6): 913-5.

[21] Breiteneder H, Mills C. Nonspecific lipid-transfer proteins in plant foods and pollens: an important allergen class. Curr Opin Allergy Clin Immunol 2005; 5(3): 275-9.

[22] van Loon L, van Strien E. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol Mol Plant Pathol 1999; 55: 85-97.

[23] Asero R, Mistrello G, Roncarolo D, *et al*. Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. Int Arch Allergy Immunol 2000; 122(1): 20-32.

[24] van Ree R. Clinical importance of non-specific lipid transfer proteins as food allergens. Biochem Soc Trans 2002; 30(Pt 6): 910-3.

[25] Fernandez-Rivas M, Gonzalez-Mancebo E, Rodriguez-Perez R, *et al*. Clinically relevant peach allergy is related to peach lipid transfer protein, Pru p 3, in the Spanish population. J Allergy Clin Immunol 2003; 112(4): 789-95.

[26] Garcia-Selles FJ, Diaz-Perales A, Sanchez-Monge R, *et al*. Patterns of reactivity to lipid transfer proteins of plant foods and Artemisia pollen: an *in vivo* study. Int Arch Allergy Immunol 2002; 128(2): 115-22.

[27] Ebner C, Birkner T, Valenta R, *et al*. Common epitopes of birch pollen and apples--studies by western and northern blot. J Allergy Clin Immunol 1991; 88(4): 588-94.

[28] Scheurer S, Son DY, Boehm M, *et al*. Cross-reactivity and epitope analysis of Pru a 1, the major cherry allergen. Mol Immunol 1999; 36(3): 155-67.

[29] Lombardero M, Garcia-Selles FJ, Polo F, *et al*. Prevalence of sensitization to Artemisia allergens Art v 1, Art v 3 and Art v 60 kDa. Cross-reactivity among Art v 3 and other relevant lipid-transfer protein allergens. Clin Exp Allergy 2004; 34(9): 1415-21.

[30] Pastorello EA, Pravettoni V, Farioli L, *et al*. Hypersensitivity to mugwort (Artemisia vulgaris) in patients with peach allergy is due to a common lipid transfer protein allergen and is often without clinical expression. J Allergy Clin Immunol 2002; 110(2): 310-7.

[31] Salcedo G, Sanchez-Monge R, Diaz-Perales A, Garcia-Casado G, Barber D. Plant non-specific lipid transfer proteins as food and pollen allergens. Clin Exp Allergy 2004; 34(9): 1336-41.

[32] Vassilopoulou E, Zuidmeer L, Akkerdaas J, *et al*. Severe immediate allergic reactions to grapes: part of a lipid transfer protein-associated clinical syndrome. Int Arch Allergy Immunol 2007; 143(2): 92-102.

[33] van der Maarel MJ, van der Veen B, Uitdehaag JC, Leemhuis H, Dijkhuizen L. Properties and applications of starch-converting enzymes of the alpha-amylase family. J Biotechnol 2002; 94(2): 137-55.

[34] Dunwell JM, Purvis A, Khuri S. Cupins: the most functionally diverse protein superfamily? Phytochemistry 2004; 65(1): 7-17.

[35] Asensio T, Crespo JF, Sanchez-Monge R, *et al*. Novel plant pathogenesis-related protein family involved in food allergy. J Allergy Clin Immunol 2004; 114(4): 896-9.

[36] Kasprzewska A. Plant chitinases--regulation and function. Cell Mol Biol Lett 2003; 8(3): 809-24.

[37] O'Riordain G, Radauer C, Hoffmann-Sommergruber K, *et al*. Cloning and molecular characterization of the Hevea brasiliensis allergen Hev b 11, a class I chitinase. Clin Exp Allergy 2002; 32(3): 455-62.

[38] Karisola P, Kotovuori A, Poikonen S, *et al*. Isolated hevein-like domains, but not 31-kd endochitinases, are responsible for IgE-mediated *in vitro* and *in vivo* reactions in latex-fruit syndrome. J Allergy Clin Immunol 2005; 115(3): 598-605.

[39] Posch A, Chen Z, Wheeler C, Dunn MJ, Raulf-Heimsoth M, Baur X. Characterization and identification of latex allergens by two-dimensional electrophoresis and protein microsequencing. J Allergy Clin Immunol 1997; 99(3): 385-95.

[40] Diaz-Perales A, Collada C, Blanco C, *et al*. Cross-reactions in the latex-fruit syndrome: A relevant role of chitinases but not of complex asparagine-linked glycans. J Allergy Clin Immunol 1999; 104(3 Pt 1): 681-7.

[41] Diaz-Perales A, Blanco C, Sanchez-Monge R, Varela J, Carrillo T, Salcedo G. Analysis of avocado allergen (Prs a 1) IgE-binding peptides generated by simulated gastric fluid digestion. J Allergy Clin Immunol 2003; 112(5): 1002-7.

[42] Hanninen AR, Mikkola JH, Kalkkinen N, *et al*. Increased allergen production in turnip (Brassica rapa) by treatments activating defense mechanisms. J Allergy Clin Immunol 1999; 104(1): 194-201.

[43] Breiteneder H. Thaumatin-like proteins -- a new family of pollen and fruit allergens. Allergy 2004; 59(5): 479-81.

[44] Gajhede M, Osmark P, Poulsen FM, *et al*. X-ray and NMR structure of Bet v 1, the origin of birch pollen allergy. Nat Struct Biol 1996; 3(12): 1040-5.

[45] Neudecker P, Schweimer K, Nerkamp J, *et al*. Allergic cross-reactivity made visible: solution structure of the major cherry allergen Pru av 1. J Biol Chem 2001; 276(25): 22756-63.

[46] Schirmer T, Hoffimann-Sommergrube K, Susani M, Breiteneder H, Markovic-Housley Z. Crystal structure of the major celery allergen Api g 1: molecular analysis of cross-reactivity. J Mol Biol 2005; 351(5): 1101-9.

[47] Markovic-Housley Z, Degano M, Lamba D, *et al*. Crystal structure of a hypoallergenic isoform of the major birch pollen allergen Bet v 1 and its likely biological function as a plant steroid carrier. J Mol Biol 2003; 325(1): 123-33.

[48] Mogensen JE, Ferreras M, Wimmer R, Petersen SV, Enghild JJ, Otzen DE. The major allergen from birch tree pollen, Bet v 1, binds and permeabilizes membranes. Biochemistry 2007; 46(11): 3356-65.

[49] Park CJ, Kim KJ, Shin R, Park JM, Shin YC, Paek KH. Pathogenesis-related protein 10 isolated from hot pepper functions as a ribonuclease in an antiviral pathway. Plant J 2004; 37(2): 186-98.

[50] Shakib F, Schulz O, Sewell H. A mite subversive: cleavage of CD23 and CD25 by Der p 1 enhances allergenicity. Immunol Today 1998; 19(7): 313-6.

[51] Wan H, Winton HL, Soeller C, *et al*. Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. J Clin Invest 1999; 104(1): 123-33.

[52] Holmgren A. Thioredoxin structure and mechanism: conformational changes on oxidation of the active-site sulfhydryls to a disulfide. Structure 1995; 3(3): 239-43.

[53] Stammers DK, Ren J, Leslie K, *et al*. The structure of the negative transcriptional regulator NmrA reveals a structural superfamily which includes the short-chain dehydrogenase/reductases. Embo J 2001; 20(23): 6619-26.

[54] Bolhaar ST, van Ree R, Ma Y, *et al*. Severe allergy to sharon fruit caused by birch pollen. Int Arch Allergy Immunol 2005; 136(1): 45-52.

[55] Karamloo F, Wangorsch A, Kasahara H, *et al*. Phenylcoumaran benzylic ether and isoflavonoid reductases are a new class of cross-reactive allergens in birch pollen, fruits and vegetables. Eur J Biochem 2001; 268(20): 5310-20.

[56] Vieths S, Frank E, Scheurer S, Meyer HE, Hrazdina G, Haustein D. Characterization of a new IgE-binding 35-kDa protein from birch pollen with cross-reacting homologues in various plant foods. Scand J Immunol 1998; 47(3): 263-72.

[57] Horvathova V, Janecek S, Sturdik E. Amylolytic enzymes: molecular aspects of their properties. Gen Physiol Biophys 2001; 20(1): 7-32.

[58] Kondo Y, Urisu A, Tokuda R. Identification and characterization of the allergens in the tomato fruit by immunoblotting. Int Arch Allergy Immunol 2001; 126(4): 294-9.

[59] Kitagawa M, Moriyama T, Ito H, Ozasa S, Adachi A, Yasuda J, *et al*. Reduction of allergenic proteins by the effect of the ripening inhibitor (rin) mutant gene in an F1 hybrid of the rin mutant tomato. Biosci Biotechnol Biochem 2006; 70(5): 1227-33.

[60] Lee MF, Hwang GY, Chen YH, Lin HC, Wu CH. Molecular cloning of Indian jujube (Zizyphus mauritiana) allergen Ziz m 1 with sequence similarity to plant class III chitinases. Mol Immunol 2006; 43(8): 1144-51.

[61] Seppala U, Palosuo T, Seppala U, *et al*. IgE reactivity to patatin-like latex allergen, Hev b 7, and to patatin of potato tuber, Sol t 1, in adults and children allergic to natural rubber latex. Allergy 2000; 55(3): 266-73.

[62] Coulombe R, Li Y, Takebe S, *et al*. Crystallization and preliminary X-ray diffraction studies of human procathepsin L. Proteins 1996; 25(3): 398-400.

[63] Mills EN, Jenkins JA, Alcocer MJ, Shewry PR. Structural, biological, and evolutionary relationships of plant food allergens sensitizing *via* the gastrointestinal tract. Crit Rev Food Sci Nutr 2004; 44(5): 379-407.

[64] Siezen RJ, Leunissen JA. Subtilases: the superfamily of subtilisin-like serine proteases. Protein Sci 1997; 6(3): 501-23.

[65] Shen HD, Tam MF, Chou H, Han SH. The importance of serine proteinases as aeroallergens associated with asthma. Int Arch Allergy Immunol 1999; 119(4): 259-64.

[66] Facchini PJ, Penzes C, Johnson AG, Bull D. Molecular characterization of berberine bridge enzyme genes from opium poppy. Plant Physiol 1996; 112(4): 1669-77.

[67] Dewitt AM, Andersson K, Peltre G, Lidholm J. Cloning, expression and immunological characterization of full-length timothy grass pollen allergen Phl p 4, a berberine bridge enzyme-like protein with homology to celery allergen Api g 5. Clin Exp Allergy 2006; 36(1): 77-86.

[68] Aalberse RC, Koshte V, Clemens JG. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. J Allergy Clin Immunol 1981; 68(5): 356-64.

[69] Aalberse RC, van Ree R. Crossreactive carbohydrate determinants. Clin Rev Allergy Immunol 1997 Win; 15(4): 375-87.

[70] van Ree R. Carbohydrate epitopes and their relevance for the diagnosis and treatment of allergic diseases. Int Arch Allergy Immunol 2002; 129(3): 189-97.

[71] Abrahamson M. Cystatins. Methods Enzymol 1994; 244: 685-700.

[72] Nagata K, Kudo N, Abe K, Arai S, Tanokura M. Three-dimensional solution structure of oryzacystatin-I, a cysteine proteinase inhibitor of the rice, Oryza sativa L. japonica. Biochemistry 2000; 39(48): 14753-60.

[73] Martinez M, Abraham Z, Gambardella M, Echaide M, Carbonero P, Diaz I. The strawberry gene Cyf1 encodes a phytocystatin with antifungal properties. J Exp Bot 2005; 56(417): 1821-9.

[74] Abe K, Emori Y, Kondo H, Suzuki K, Arai S. Molecular cloning of a cysteine proteinase inhibitor of rice (oryzacystatin). Homology with animal cystatins and transient expression in the ripening process of rice seeds. J Biol Chem 1987; 262(35): 16793-7.

[75] Rogers BL, Pollock J, Klapper DG, Griffith IJ. Sequence of the proteinase-inhibitor cystatin homologue from the pollen of Ambrosia artemisiifolia (short ragweed). Gene 1993; 133(2): 219-21.

[76] Burks AW, Cockrell G, Connaughton C, Guin J, Allen W, Helm RM. Identification of peanut agglutinin and soybean trypsin inhibitor as minor legume allergens. Int Arch Allergy Immunol 1994; 105(2): 143-9.

[77] Moroz LA, Yang WH. Kunitz soybean trypsin inhibitor: a specific allergen in food anaphylaxis. N Engl J Med 1980; 302(20): 1126-8.

[78] Gu X, Beardslee T, Zeece M, Sarath G, Markwell J. Identification of IgE-binding proteins in soy lecithin. Int Arch Allergy Immunol 2001; 126(3): 218-25.

[79] Roychaudhuri R, Sarath G, Zeece M, Markwell J. Stability of the allergenic soybean Kunitz trypsin inhibitor. Biochim Biophys Acta 2004; 1699(1-2): 207-12.

[80] Ramachandran S, Christensen HE, Ishimaru Y, *et al*. Profilin plays a role in cell elongation, cell shape maintenance, and flowering in Arabidopsis. Plant Physiol 2000; 124(4): 1637-47.

[81] Valster AH, Pierson ES, Valenta R, Hepler PK, Emons A. Probing the Plant Actin Cytoskeleton during Cytokinesis and Interphase by Profilin Microinjection. Plant Cell 1997; 9(10): 1815-24.

[82] Valenta R, Duchene M, Ebner C, *et al*. Profilins constitute a novel family of functional plant pan-allergens. J Exp Med 1992; 175(2): 377-85.

[83] Witke W. The role of profilin complexes in cell motility and other cellular processes. Trends Cell Biol 2004; 14(8): 461-9.

[84] Sankian M, Varasteh A, Pazouki N, Mahmoudi M. Sequence homology: a poor predictive value for profilins cross-reactivity. Clin Mol Allergy 2005; 3: 13.

[85] Wopfner N, Gadermaier G, Egger M, *et al*. The spectrum of allergens in ragweed and mugwort pollen. Int Arch Allergy Immunol 2005; 138(4): 337-46.

[86] Asero R, Mistrello G, Roncarolo D, *et al*. Detection of clinical markers of sensitization to profilin in patients allergic to plant-derived foods. J Allergy Clin Immunol 2003; 112(2): 427-32.

[87] Rodriguez-Perez R, Crespo JF, Rodriguez J, Salcedo G. Profilin is a relevant melon allergen susceptible to pepsin digestion in patients with oral allergy syndrome. J Allergy Clin Immunol 2003; 111(3): 634-9.

[88] Ballmer-Weber BK, Vieths S, Luttkopf D, Heuschmann P, Wuthrich B. Celery allergy confirmed by double-blind, placebo-controlled food challenge: a clinical study in 32 subjects with a history of adverse reactions to celery root. J Allergy Clin Immunol 2000; 106(2): 373-8.

[89] Jankiewicz A, Aulepp H, Baltes W, *et al*. Allergic sensitization to native and heated celery root in pollen-sensitive patients investigated by skin test and IgE binding. Int Arch Allergy Immunol 1996; 111(3): 268-78.

[90] Valenta R, Duchene M, Pettenburger K, *et al*. Identification of profilin as a novel pollen aller; IgE autoreactivity in sensitized individuals. Science 1991; 253(5019): 557-60.

[91] van Ree R, Fernandez-Rivas M, Cuevas M, van Wijngaarden M, Aalberse RC. Pollen-related allergy to peach and apple: an important role for profilin. J Allergy Clin Immunol 1995; 95(3): 726-34.

[92] Fah J, Wuthrich B, Vieths S. Anaphylactic reaction to lychee fruit: evidence for sensitization to profilin. Clin Exp Allergy 1995; 25(10): 1018-23.

[93] Willerroider M, Fuchs H, Ballmer-Weber BK, *et al*. Cloning and molecular and immunological characterisation of two new food allergens, Cap a 2 and Lyc e 1, profilins from bell pepper (Capsicum annuum) and Tomato (Lycopersicon esculentum). Int Arch Allergy Immunol 2003; 131(4): 245-55.

[94] Capuano F, Beaudoin F, Napier JA, Shewry PR. Properties and exploitation of oleosins. Biotechnol Adv 2007; 25(2): 203-6.

[95] Leduc V, Moneret-Vautrin DA, Tzen JT, Morisset M, Guerin L, Kanny G. Identification of oleosins as major allergens in sesame seed allergic patients. Allergy 2006; 61(3): 349-56.

[96] Tamburrini M, Cerasuolo I, Carratore V, *et al*. Kiwellin, a novel protein from kiwi fruit. Purification, biochemical characterization and identification as an allergen*. Protein J 2005; 24(7-8): 423-9.

[97] Bohle B, Vieths S. Improving diagnostic tests for food allergy with recombinant allergens. Methods 2004; 32(3): 292-9.

[98] Schimek EM, Zwolfer B, Briza P, *et al*. Gastrointestinal digestion of Bet v 1-homologous food allergens destroys their mediator-releasing, but not T cell-activating, capacity. J Allergy Clin Immunol 2005; 116(6): 1327-33.

[99] Emanuelsson C, Spangfort MD. Allergens as eukaryotic proteins lacking bacterial homologues. Mol Immunol 2007; 44(12): 3256-60.

Received: November 16, 2007 Revised: December 20, 2007 Accepted: December 31, 2007