

Food Allergens: Molecular and Immunological Characteristics

Heimo Breiteneder and E.N. Clare Mills

KEY CONCEPTS

- Food allergens belong to a limited number of protein families with different molecular properties, which may mean routes of sensitization differ for different allergen families.
- Food allergens that sensitize via the gastrointestinal tract have molecular features that enhance stability to thermal and proteolytical denaturation.
- Two allergen families (caseins and cupins) thought to sensitize via the gastrointestinal tract retain their allergenicity even after digestion for reasons which are not understood.
- Plant food allergens related to pollen allergens generally have no stability-enhancing characteristics and only induce oral allergy syndrome (OAS) as a secondary reaction to primary sensitization to pollen.

Introduction

The post-genomic era, with its explosion of information about protein and genome sequences, is allowing us to study molecular relationships in new ways, and notably within the context of evolution. Allergenic proteins have not suddenly appeared on the protein landscape but are the result of a long chain of formative processes that resulted in the creation of protein architectures that are treated as allergenic by an atopic immune system. Allergens are restricted to a very small number of protein families which share characteristic three-dimensional structures or scaffolds, as has been shown for plant food [1] and pollen allergens [2]. For some of these protein scaffolds, their origins can be followed back today to even archaebacteria as has been done for the cupin superfamily [3]. Although most members of the prolamin superfamily seem to be restricted to the seeds of dicotyledonous plants [4], proteins related to 2S albumins have been identified in the spores of ostrich fern [5]. The common ancestors of ferns and angiosperms lived more than 300 million years ago. Some of the evolved structures have been proven so successful that they are conserved between plants and animals as is the case for thaumatin-like

proteins [6]. Consequently, allergenicity seems to be linked to certain structural features of molecules that are members of a limited number of protein families. In general, within a given protein family, those known to be allergens represent only a fraction of its members, which has been well demonstrated for the Bet v 1 family of proteins [7]. In addition to any intrinsic allergenicity of the protein scaffold, this also may relate to issues of exposure, with some forms not being found in edible tissues or transiently expressed during development such that levels in the exposing agent are low. The most important food allergen families will be discussed in this chapter.

Food allergen protein families

Based on their shared amino acid sequences and conserved three-dimensional structures, proteins can be classified into families using various bioinformatics tools which form the basis of several protein family databases, one of which is Pfam [8]. Over the past 10 years or so there has been an explosion in the numbers of well characterized allergens, which have been sequenced and are being collected into a number of databases to facilitate bioinformatic analysis [9]. We have undertaken this analysis for both plant [1] and animal food allergens [10] along with pollen allergens [2]. They show similar distributions with the majority of allergens in each group falling into just 3–12 families with a tail

of between 14 and 23 families comprising between 1 and 3 allergens each. With regards to food allergens, around 65% of those from plants belong to just four protein families, the prolamin, cupin, Bet v 1-like, and profilin families [1], supporting molecular and structural approaches to the classification of plant food allergens [4,11]. Similarly, animal food allergens can be classified into three main families, the tropomyosins, EF-hand proteins, and caseins [10]. Such patterns of behavior beg the question of why certain protein scaffolds dominate the landscape of allergen structures? Are there structural features that predispose certain proteins to becoming allergens? Certainly detailed analysis of the secondary structural elements in proteins has not shown any relationship with allergenicity [12], but protein structure–function relationships can be very subtle. Using the food allergen family classification, we will summarize the properties of known food allergens and discuss how their structures and properties might determine their allergenic potency.

Food allergens of animal origin (Table 4.1)

Tropomyosins

Tropomyosins are a family of closely related proteins present in muscle and non-muscle cells [13]. Together with actin and myosin, tropomyosins play a key regulatory role in muscle contraction. Tropomyosins contain 40 uninterrupted heptapeptide repeats and are two-stranded proteins that occur as α -helical coiled coils [14]. Tropomyosins form head-to-tail polymers along the length of an actin filament [15] and are the major allergens of two invertebrate groups, Crustacea and Mollusca, that are generally referred to as shellfish. Shrimp, crab, squid, and abalone are assumed to be largely responsible for seafood allergies. Tropomyosins were originally identified as major shrimp allergens by several laboratories [16–18] and today they are recognized as invertebrate pan-allergens [19]. The first two residues of the IgE-binding region (epitope) in the C-terminal portion of the protein appear to be crucial for IgE binding and is not found in vertebrate tropomyosin. As a consequence of the lack of homology in the IgE epitopes, there is no cross-reactivity of IgE from shellfish-allergic individuals to animal muscle tropomyosins. Allergenic tropomyosins are heat stable and cross-reactive between the various crustacean and mollusk species [20]. Extracts from boiled *Penaeus indicus* shrimp contained Pen i 1 with unaltered allergenicity [21]. Water-soluble shrimp allergens were also detected in the cooking water after boiling [22].

Parvalbumins

The second largest animal food allergen family are the parvalbumins. Abundant in the white muscle of many fish species, parvalbumins are characterized by the possession of a

widely found calcium-binding domain which is known as the EF-hand [23]. The EF-hand is a motif that consists of a loop of 12 amino acid residues that is flanked on either side by a 12-residue α -helical domain. Parvalbumins comprise three such domains [24], two of which are able to bind calcium [25]. Parvalbumins are important for the relaxation of muscle fibers by binding free intracellular calcium [26]. The binding of the calcium ligands is necessary for the correct parvalbumin conformation, loss of calcium resulting in a large change in conformation with an associated loss of IgE-binding capacity [27,28]. Parvalbumins with bound calcium ions possess a remarkable stability to denaturation by heat [29]. The ability to act as major fish allergens is obviously linked to the stability of parvalbumins to heat, denaturing chemicals, and proteolytic enzymes [30]. Parvalbumins can be subdivided into two distinct evolutionary lineages, the α - and the β -parvalbumins, although their overall architectures are very similar. The β -parvalbumins are generally allergenic. Gad c 1, a codfish allergen, was the first allergenic fish parvalbumin that was purified and characterized [31,32]. Today, allergenic β -parvalbumins have been characterized from many different fish species and are considered as pan-allergens in fish [33]. The cross-reactivity of fish and frog muscle in fish-allergic individuals has been attributed to the structural similarities between their parvalbumins [34]. An α -parvalbumin of frog has also been described as allergenic [35].

Caseins

Structurally mobile proteins, they are found in mammalian milk at a concentration of around 15 mg/ml and are responsible for binding calcium through clusters of phosphoserine and/or phosphothreonine residues in α_{s1} -, α_{s2} -, and β -caseins although the α_{s2} -casein gene is not expressed in man. The casein polypeptides form a shell around amorphous calcium phosphate to form microstructures called nanoclusters allowing calcium levels in milk to exceed the solubility limit of calcium phosphate. The nanoclusters are assembled into the casein micelles found in milk which are in turn stabilized by κ -casein [36]. The α - and β -caseins are related to the secretory calcium-binding phosphoprotein family together with proteins involved in mineralization and salivary proteins whilst κ -caseins may be distantly related to fibrinogen γ -chain [37]. Caseins are a major food allergen involved in cow's milk allergy, which affects predominantly young children. Studies on the IgE cross-reactivity between different types of casein in a group of cow's milk-allergic infants found that 90% had serum IgE against α_{s2} -casein, 55% against α_{s1} -casein, whilst only 15% had IgE against β -casein [38]. This pattern of reactivity appears to be related to the degree of similarity between bovine and human casein with caseins least like human caseins being more reactive. Thus, bovine β -casein appears to be the least reactive and has the highest identity to human casein of 53% whilst bovine α_{s2} -caseins were the

Table 4.1 Food allergens of animal origin

Animal food allergen family	Function	Source	Allergen name	Sequence accession	Reference
Tropomyosin superfamily	Tropomyosins bind to actin in muscle increasing thin filament stability and rigidity. It may play an important role with troponin in controlling muscle contraction	Brown shrimp (<i>Farfantepenaeus aztecus</i>)	Pen a 1	AAZ76743	[16]
		Greasy backed shrimp (<i>Metapenaeus ensis</i>)	Met e 1	Q25456	[39]
		Black tiger shrimp (<i>Penaeus monodon</i>)	Pen m 1	Not known	[40]
		Indian prawn (<i>Fenneropenaeus indicus</i>)	Pen i 1	Peptides only	[16]
		Snail (<i>Helix aspersa</i>)	Hel as 1	O97192	[41]
		Squid (<i>Todarodes pacificus</i>)	Tod p 1	Peptides only	[42]
		Oyster (<i>Crassostrea gigas</i>)	Cra g 1 Cra g 2 Cra g 1.03	Q95WY0	[43]
		Crab (<i>Charybdis feriatus</i>)	Cha f 1	Q9N2R3	[44]
		Abalone (<i>Haliotis diversicolor</i>)	Hal d 1	Q9GZ71	[45]
		Cod (<i>Gadus morhua</i>)	Gad c 1	Q90YK9, Q90YL0	[46]
Parvalbumin superfamily	Parvalbumins control the flow of calcium from troponin C back to membrane-bound pumps after a muscle contraction	Carp (<i>Cyprinus carpio</i>)	Cyp c 1.01 Cyp c 1.02	Q8UUS3 Q8UUS2	[47]
		Salmon (<i>Salmo salar</i>)	Sal s 1.01 Sal s 1.02	Q91482 Q91483	[48]
		Tuna (<i>Thunnus tonggol</i>)	Thu o 1.01 Thu o 1.02	None	[49]
		Edible frog (<i>Rana esculenta</i>)	Ran e 1	Q8JIU2 P02627	[35]
			Ran e 2	Q8JIU1, P02617	
Caseins	Caseins form stable micellar calcium phosphate protein complexes in mammalian milks	Domestic cow (<i>Bos taurus</i>)	Bos d 8		[50]
			α_{s1n}	P02662	
			α_{s2}	P02663	
			β -casein	P02666	
			κ -casein	P02668	
		Goat (<i>Capra hircus</i>)	α_{s1n}	P18626	[51]
			α_{s2}	P33049	
			β -casein	P33048	
			κ -casein	P02670	
		Sheep (<i>Ovis aries</i>)	α_{s1n}	P04653	[52]
			α_{s2}	P04654	
			β -casein	P11839	
			κ -casein	P02669	

most reactive being least similar with only ~16% identity to the human homolog. There is considerable similarity in the caseins from different mammalian milks used for human consumption, which explains their IgE cross-reactivity. It has been observed that cow's milk-allergic patients generally react to goat's milk on oral challenge [51], whose caseins have sequence identities of over 90%. However, it appears that when this sequence identity drops to between 22% and 66%, as is the case between mare's and cow's milk caseins, it is associated with tolerance, since individuals with cow's milk can tolerate mare's milk [53] and do not show IgE cross-reactivity to milk proteins from species such as camels [54].

Minor families

Lipocalins

The lipocalins are a group of diverse proteins sharing about 20% sequence identity with conserved three-dimensional structures characterized by a central tunnel which can often accommodate a diversity of lipophilic ligands [55]. They are thought to function as carriers of odorants, steroids, lipids, and pheromones amongst others. The majority of lipocalin allergens are respiratory, having been identified as the major allergens in rodent urine, animal dander, and saliva as well as in insects such as cockroaches, although the only lipocalin which acts as a food allergen is the cow's milk allergen, β -lactoglobulin [56].

Lysozyme family

Lysozyme type C and α -lactalbumins belong to the glycoside hydrolase family 22 clan of the *O*-glycosyl hydrolase superfamily and have probably evolved from a common ancestral protein. However, they have distinctly different functions; α -lactalbumin is involved in lactose synthesis in milk, and lysozyme acts as a muramidase hydrolyzing peptidoglycans found in bacterial cell walls. Furthermore α -lactalbumin, unlike hen's egg lysozyme, binds calcium. Two food allergens belong to this clan, the minor hen's egg allergen, lysozyme (Gal d 4) and the minor cow's milk allergen, α -lactalbumin, these proteins share little sequence homology, but are superimposable three-dimensional structures [57].

Transferrin family

Transferrins are eukaryotic sulfur-rich iron-binding glycoproteins which function *in vivo* to control the level of free iron. Members of the family that have been identified as minor food allergens include milk lactotransferrin (lactoferrin) and hen egg white ovotransferrin [58,59].

Serpins

Serpins are a class of serine protease inhibitors and are found in all types of organisms with the exception of fungi and are involved in a variety of physiological processes. Many of the family members have lost their inhibitory activity [60]. Only one food allergen has been identified as belonging to this family, the hen's egg allergen ovalbumin [61].

Arginine kinases

Arginine kinases have been identified as allergens in invertebrates including food allergens such as in shrimp [40] and as cross-reactive allergens in the Indian meal moth and king prawn, lobster, and mussel [62]. This protein belongs to a family of structurally and functionally related ATP:guanido phosphotransferases that reversibly catalyze the transfer of phosphate between ATP and various phosphogens.

Ovomucoids

Kazal inhibitors which inhibit a number of serine proteases belong to a family of proteins that includes pancreatic secretory trypsin inhibitor, avian ovomucoid, and elastase inhibitor. These proteins contain between 1 and 7 Kazal-type inhibitor repeats [63]. Avian ovomucoids contain three Kazal-like inhibitory domains [64]. Chicken ovomucoid has been shown to be the dominant hen's egg white allergen Gal d 1 [61]. Gal d 1 comprises 186 amino acid residues that are arranged in three tandem domains (Gal d 1.1, Gal d 1.2, Gal d 1.3). Each domain contains three intradomain disulfide bonds. Gal d 1.1 and Gal d 1.2 contain two carbohydrate chains each, and about 50% of the Gal d 1.3 domains contain one carbohydrate chain which may act to stabilize the protein against proteolysis [65].

Food allergens of plant origin (Table 4.2)

The prolamin superfamily

The prolamin superfamily was initially defined by Kreis and co-workers [66] who observed that three groups of apparently unrelated seed proteins contained a conserved pattern of cysteine residues. These included two types of cereal seed proteins, namely the sulfur-rich prolamins and the α -amylase/trypsin inhibitors of monocotyledonous cereal seeds, together with the 2S storage albumins (Fig. 4.1(a)) found in a variety of dicotyledonous seeds including castor bean and oilseed rape. Subsequently other low molecular weight (LMW) allergenic proteins have been identified as belonging to this superfamily including soybean hydrophobic protein (Fig. 4.1(b)), non-specific lipid-transfer proteins (nsLTPs, Fig. 4.1(c)) and α -globulins. The conserved cysteine skeleton comprises a core of eight cysteine residues which includes a characteristic Cys–Cys and Cys–X–Cys motif (X representing any other residue). Two additional cysteine residues are found in the α -amylase/trypsin inhibitors. In the cereal seed storage prolamins the disulfide skeleton has been disrupted by the insertion of a repetitive domain comprising motifs rich in proline and glutamine. Whilst the way in which the disulfide connectivities formed by the cysteine residues are different in the different types of prolamin superfamily members, they share a common three-dimensional structure, examples of which are shown in Fig. 4.1. This figure illustrates the three-dimensional structures shared by these

Table 4.2 Food allergens of plant origin

Plant food allergen family	Function in plant	Botanical source	Allergen name	Sequence accession	Reference
<i>Prolamin superfamily</i> Prolamins	Seed storage protein		α - and γ -gliadin	BAA12318 BAA11251 BAA23162 Q9MSX7	[67–69]
		Apple (<i>Malus domestica</i>)	LMW glutenin Mal d 3		[70]
Non-specific lipid-transfer proteins (nsLTPs)	Function uncertain; maybe involved in transport of suberin monomers	Apricot (<i>Prunus armeniaca</i>)	Pru ar 3	G7404406	[71]
		Cherry (<i>Prunus avium</i>)	Pru av 3	AAF26449	[72]
		Peach (<i>Prunus persica</i>)	Pru p 3 (originally Pru p 1)	P81402	[73]
		Garden plum (<i>Prunus domestica</i>)	Pru d 3	P82534	[74]
		Strawberry (<i>Fragaria ananassa</i>)	Fra a 3	Q4PLT5-9 Q4PLU0	[75]
		Orange (<i>Citrus sinensis</i>)	Cit s 3	P84161	[76]
		Grape (<i>Vitis vinifera</i>)	Vit v 1	P80274	[77]
		Chestnut (<i>Castanea sativa</i>)	Cas s 8	N-terminus only	[78]
		Walnut (<i>Juglans regia</i>)	Jug r 3	Not known	[79,80]
		Hazelnut (<i>Corylus avellana</i>)	Cor a 8	AF329829	[81]
		Asparagus (<i>Asparagus officinalis</i>)	Aspa o 1		[82]
		Lettuce (<i>Lactuca sativa</i>)	Lac s 1	N-terminus only Q2A988	[83]
		Cabbage (<i>Brassica oleracea</i>)	Bra o 3	P19656	[84]
		Maize (<i>Zea mays</i>)	Zea m 14		[85]
		Barley (<i>Hordeum vulgare</i>)	Hor v 1	CAA45085	[85,86]
α -amylase/trypsin inhibitors	Provide protection against degradative proteases and amylases produced by insect pests and pathogens	Rice (<i>Oryza sativa</i>)	Rag 1, 2 5, 5.b, 14, 14b, 16, 17	Q01881 S59922 S59924 S59925 BAA01997 BAA01998 BAA01999 BAA02000	[87–89]
		Walnut (<i>Juglans regia</i>)	Jug r 1	JRU66866	[90]
		Almond (<i>Prunus dulcis</i>)		P82944	[91]
		Brazil nut (<i>Bertholletia excelsa</i>)	Ber e 1	CAA38362	[92]
		Cashew nut (<i>Anacardium occidentale</i>)	Ana o 3	Q8H2B8	[93]
		White mustard (<i>Sinapis alba</i>)	Sin a 1	P15322	[94]
		Black mustard (<i>Brassica juncea</i>)	Bra j 1	P80207	[95]
					(Continued)
2S albumins	Seed storage protein				

Table 4.2 (Continued)

Plant food allergen family	Function in plant	Botanical source	Allergen name	Sequence accession	Reference
Bet v 1 superfamily		Chickpea (<i>Cicer arietinum</i>)	None	None	[96]
		Peanut (<i>Arachis hypogaea</i>)	Ara h 2	L77197	[97–99]
			Ara h 6	AF091737	
			Ara h 7	AF092846	
		Sesame (<i>Sesamum indicum</i>)	Ses i 1	AAD42943	[100]
			Ses i 2	Q9XHP1	[101]
		Sunflower (<i>Helianthus annuus</i>)	SFA-8		[102]
		Apple (<i>Malus domestica</i>)	Mal d 1	P43211	[103]
		Pear (<i>Pyrus communis</i>)	Pyr c 1	O65200	[104]
		Cherry (<i>Prunus avium</i>)	Pru av 1 (originally Pru a 1)	O24248	[105]
Cupin superfamily 7S (vicilin like) Globulins	Seed storage protein	Strawberry (<i>Fragaria ananassa</i>)		Q6QHU3	
		Carrot (<i>Daucus carota</i>)		Q6QHU2	
		Celery root (<i>Apium graveolens</i>)		AY679601	[106]
			Dau c 1	O04298	[107]
			Api g 1	P49372	[108]
				P92918	
		Hazelnut (<i>Corylus avellana</i>)	Cor a 1.04	Q9FPK2,3,4	[109]
				Q9SWR4	
		Soy (<i>Glycine max</i>)	Gly m 4	P26987	[110]
		Peanut (<i>Arachis hypogaea</i>)	Conarachin, Ara h 1	L34402	[111]
		Soy (<i>Glycine max</i>)	β-conglycinin	αP13916	[112]
				α'; P11827	
				β;P25974	
		Buckwheat (<i>Fagopyrum esculentum</i>)	BWI-1c	Q6QJL1	[113]
			BWI-2		
			BWI-2b		
			BWI-2c		
		Almond (<i>Prunus dulcis</i>)	Conglutin Gamma	P82952	[91]
		Walnut (<i>Juglans regia</i>)	Jug r 2	AAB41308	[114]
		Hazelnut (<i>Corylus avellana</i>)	Cor a 11	Q8S4P9	[115]
		Cashew nut (<i>Anacardium occidentale</i>)	Ana o	Q8L5L5	[116]
			1.0101		
			1.0102		
		Sesame (<i>Sesamum indicum</i>)	Ses i 3	Q8L5L6	
				Q9AUD0	[101]

11S (legumin like) Globulins	Seed storage protein	Peanut (<i>Arachis hypogaea</i>)	Arachin	AF093541	[97,117]
			Ara h 3	AF086821	
			Ara h 4	Gy1 (A1aBx): P04776	
			Glycinin	Gy2 (A2B1a): P04405	
	Soy (<i>Glycine max</i>)			Gy3 (AB): P11828	[118]
				Gy4 (A4/5B3): P02858	
				Gy (A3B4): P04347	
				Q9XFM4	
	Buckwheat (<i>Fagopyrum esculentum</i>)		BW24KD	O23880	[113]
			Fag e 1	O23878	
			FAGAG1		
			FA02		
Cysteine protease C 1 family	Cysteine proteases	Almond (<i>Prunus dulcis</i>)	FA18		[119,120]
			Major almond protein, amandin, prunin	Q43607	
			Ana o 2		
			Ber e 2, Excelsin		
		Cashew nut (<i>Anacardium occidentale</i>) Brazil nut (<i>Bertholletia excelsa</i>)		Q8CZP6	[121]
				Q84ND2	
		Kiwi (<i>Actinidia deliciosa</i>) Soy (<i>Glycine max</i>)			[122]
			Actinidin	Q43367	
			Gly m Bd 30K; P34;	P22895	
			Gly m 1		

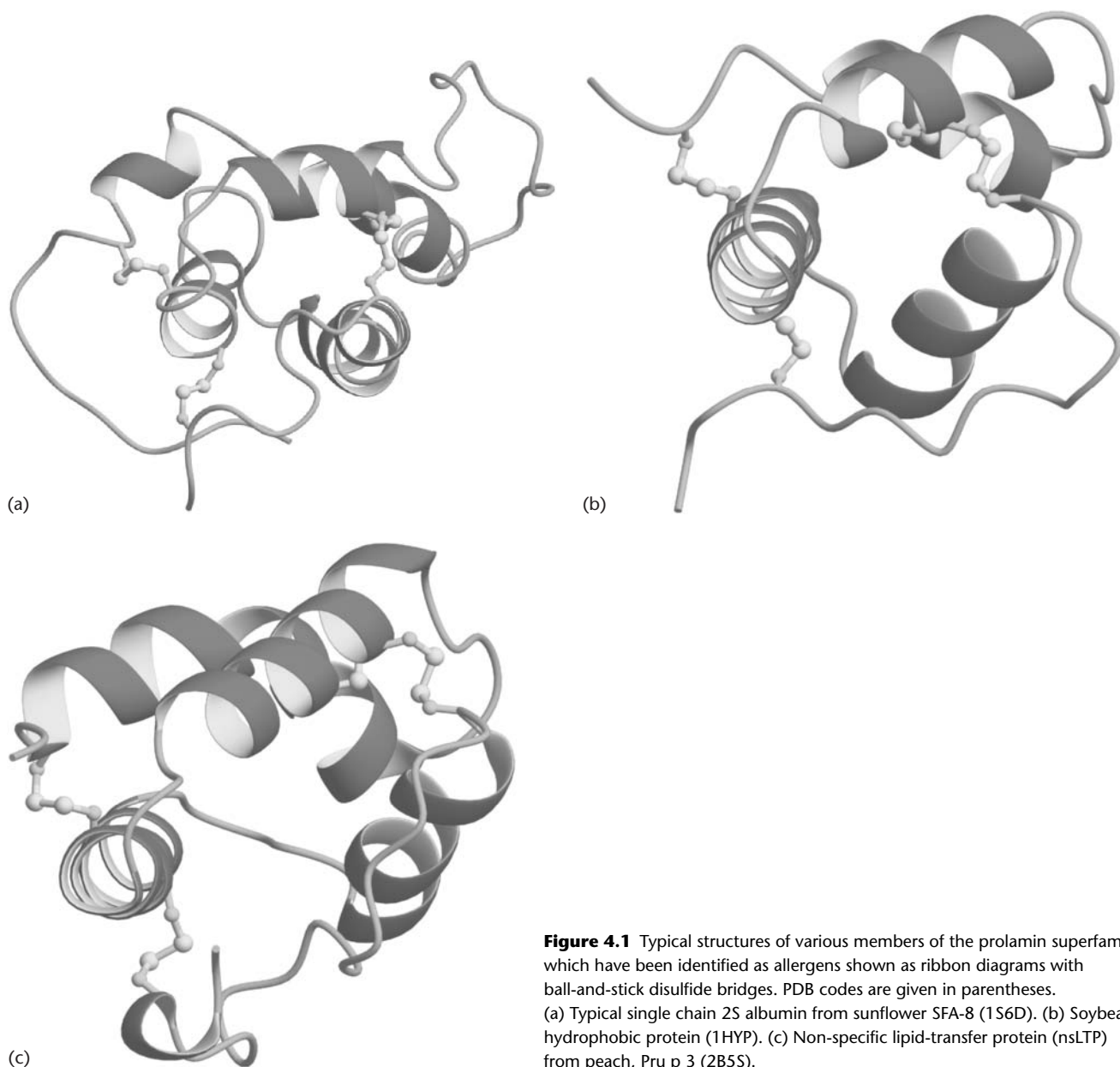


Figure 4.1 Typical structures of various members of the prolamin superfamily which have been identified as allergens shown as ribbon diagrams with ball-and-stick disulfide bridges. PDB codes are given in parentheses.

(a) Typical single chain 2S albumin from sunflower SFA-8 (1S6D). (b) Soybean hydrophobic protein (1HYP). (c) Non-specific lipid-transfer protein (nsLTP) from peach, Pru p 3 (2B5S).

proteins, which consist of bundles of four α -helices stabilized by disulfide bonds which are arranged in such a way as to create a lipid-binding tunnel in the nsLTPs, which is collapsed in the 2S albumin structures. As yet no ancestral type has been identified, and consequently this scaffold appears to be found almost exclusively in flowering plants.

The lipid-binding tunnel of the nsLTPs shows considerable flexibility being able to accommodate a diverse range of lipophiles including prostaglandins [124] and up to two fatty acids lying side by side [125]. Apart from the seed storage prolamins, whose properties are dominated by the inserted repetitive domain, the physicochemical properties of prolamin superfamily members are dominated by their intramolecular disulfide bonds. Compact proteins, the disulfide

bonds in the prolamin superfamily members, are responsible for maintaining the three-dimensional structure even after heating, which is associated with retaining their allergenic properties after cooking [126]. Their structure and IgE-binding properties are only being altered if severe heating results in hydrolysis of these bonds [127]. These same structural attributes underlie their resistance to proteolysis, with several members, including the 2S albumins [128] and nsLTP allergens [129] being highly resistant to gastric and duodenal digestion. Any degradation that does occur appears to leave the major IgE-binding sites intact explaining the fact that simulated gastrointestinal digestion does not alter their ability to elicit skin reactions *in vivo*, as has been observed for the grape nsLTP [130].

Cereal prolamins

As a consequence of the inserted repetitive domain, the α -helical structure has been disrupted in the seed storage prolamins. Their properties are dominated by the repetitive domain which is thought to adopt an ensemble of unfolded and secondary structures comprising overlapping β -turns or poly-L-proline II structures which may form a loose spiral structure [131]. They comprise around half of the protein found in grain from the related cereals, wheat, barley, and rye, those from wheat being able to form large disulfide-linked polymers which comprise the viscoelastic protein fraction known as gluten. These proteins are characteristically insoluble in dilute salt solutions, either in the native state or after reduction of inter-chain disulfide bonds, being soluble instead in aqueous alcohols. In addition to their role in triggering celiac disease, several types of cereal storage prolamins have been identified as triggering IgE-mediated allergies including γ -, α -, and ω -5 gliadins [67,68,132] in addition to the polymeric HMW and LMW subunits of glutenin [69,133–135]. Cooking appears to affect allergenicity and one study suggested that baking may be essential for allergenicity of cereal prolamins [136].

Bifunctional inhibitors

The other group of prolamins superfamily allergens unique to cereals are the α -amylase/trypsin inhibitors which have been found to sensitize individuals via the lungs resulting in occupational allergies to wheat flour such as Baker's asthma or via the gastrointestinal tract for cereal-containing foods including wheat, barley, and rice. They were initially identified in extracts made with chloroform/water mixtures (and hence called CM proteins) but are also soluble in water, dilute saline, or alcohol/water mixtures. More detailed studies have revealed a range of monomeric, dimeric, and tetrameric forms, many of the subunits being glycosylated [137]. The individual subunits are either inactive or inhibitory to trypsin (and sometimes other proteinases), α -amylases from insects (including pests) or both enzymes (i.e. the inhibitors are bifunctional). The best characterized allergens are the α -amylase inhibitors of rice grain [87], although there is one report of a $M_r \sim 15,000$ subunit being involved in wheat allergy [138]. Allergens with M_r of 16,000 have also been characterized in corn and beer (originating from barley) which appear to belong to the α -amylase inhibitor family [85,86].

2S albumins

The 2S albumins are a major family of storage proteins [139] and appear to be restricted to seeds of dicotyledonous plants where they may accompany the cupin globulin seed storage proteins (see below). Most 2S albumins are synthesized as single chains of M_r 10,000–15,000 which, depending on the plant species, may be post-translationally processed to give small and large subunits that usually remain joined by

disulfide bonds. In some plant species such as peanut and sunflower, the precursors are unprocessed and remain as a single polypeptide chain (Fig. 4.1(a)). 2S albumins can act as both occupational (sensitizing through inhalation of dusts) and food allergens, having been identified as the major allergenic components of many foods including the peanut allergens Ara h 2, 6, and 7 [97,98], oriental and yellow mustard allergens Bra j 1 and Sin a 1 [94,95], the walnut allergen Jug r 1 [90], Ses i 1 and 2 from sesame [100,101], Ber e 1 from Brazil nut [92], and 2S albumins from almond [91] and sunflower seeds [102]. There is also some evidence that the 2S albumins of soy [140] and chickpea [96] are also allergenic.

Non-specific lipid-transfer proteins

One of the most important groups of allergens to have been identified in the last decade are the nsLTPs which appear to be involved in severe allergies to fresh fruits such as peach in the south of Europe around the Mediterranean. They have been termed as “pan-allergens” [70] and are the most widely distributed type of prolamins being found in a variety of plant organs including seeds, fruits, and vegetative tissues. Thus, in addition to being identified in many different fruits and seeds, they have also been characterized in pollen of plant species such as olive and *Parietaria judaica* [141,142]. nsLTPs as major allergens have been identified in fruits such as Pru p 3 (Fig. 4.1(c)) in peach [143], Mal d 3 in apple [70], and Vit v 1 in grape [77]. Allergenic nsLTPs have also been characterized in vegetables such as asparagus [82], cereals such as maize [85], and in a number of nuts including hazelnut [144]. As their name implies, nsLTPs were originally defined on the basis of their ability *in vitro* to transfer a range of phospholipid types from liposomes to various types of membranes such as those from mitochondria. However, this is not their *in vivo* function and it is emerging that plants have used the nsLTP scaffold in a wide range of contexts. Those involved in food allergies are found in epidermal tissues. This observation, along with their lipid-binding characteristics, has led to the view that they play a role in transporting lipids involved in the synthesis of waxy cutin and suberin layers in outer plant tissues in seeds and pollen.

The cupin superfamily

The cupins are a functionally diverse protein superfamily which has probably evolved from a prokaryotic ancestor but has not found its way into the animal kingdom. They possess a characteristic β -barrel structure, the name “cupin” being derived from Latin for barrel [3]. This basic scaffold has been utilized in a diverse range of functions including sporulation proteins in fungi, sucrose-binding activities and enzymatic activities found in germins where manganese is bound in the center of the barrel. The cupin motif has been duplicated in flowering plants to give rise to the bi-cupin 7S

and 11S globulin seed storage globulins, the three-dimensional structure of the 11S globulin of soy proglycinin is shown in Fig. 4.2(b). The 11S–12S globulins are found in the seeds of many monocotyledonous and dicotyledonous plants with homologs having been identified in gymnosperms (including conifers). They are sometimes termed legumins because they are particularly found in legume seeds and are oligomers of $M_r \sim 300,000$ – $450,000$. Each oligomer consists of six

subunits of M_r about 60,000, the products of a multigene family, non-covalently associated by intertwining α -helical regions. Each subunit is post-translationally processed to give rise to acidic (M_r about 40,000) and basic (M_r about 20,000) chains, linked by a single disulfide bond and rarely, if ever, glycosylated [145]. In contrast the 7S/8S globulins are usually trimeric proteins of M_r about 150,000–190,000, comprising subunit $M_r \sim 40,000$ –80,000, but typically about

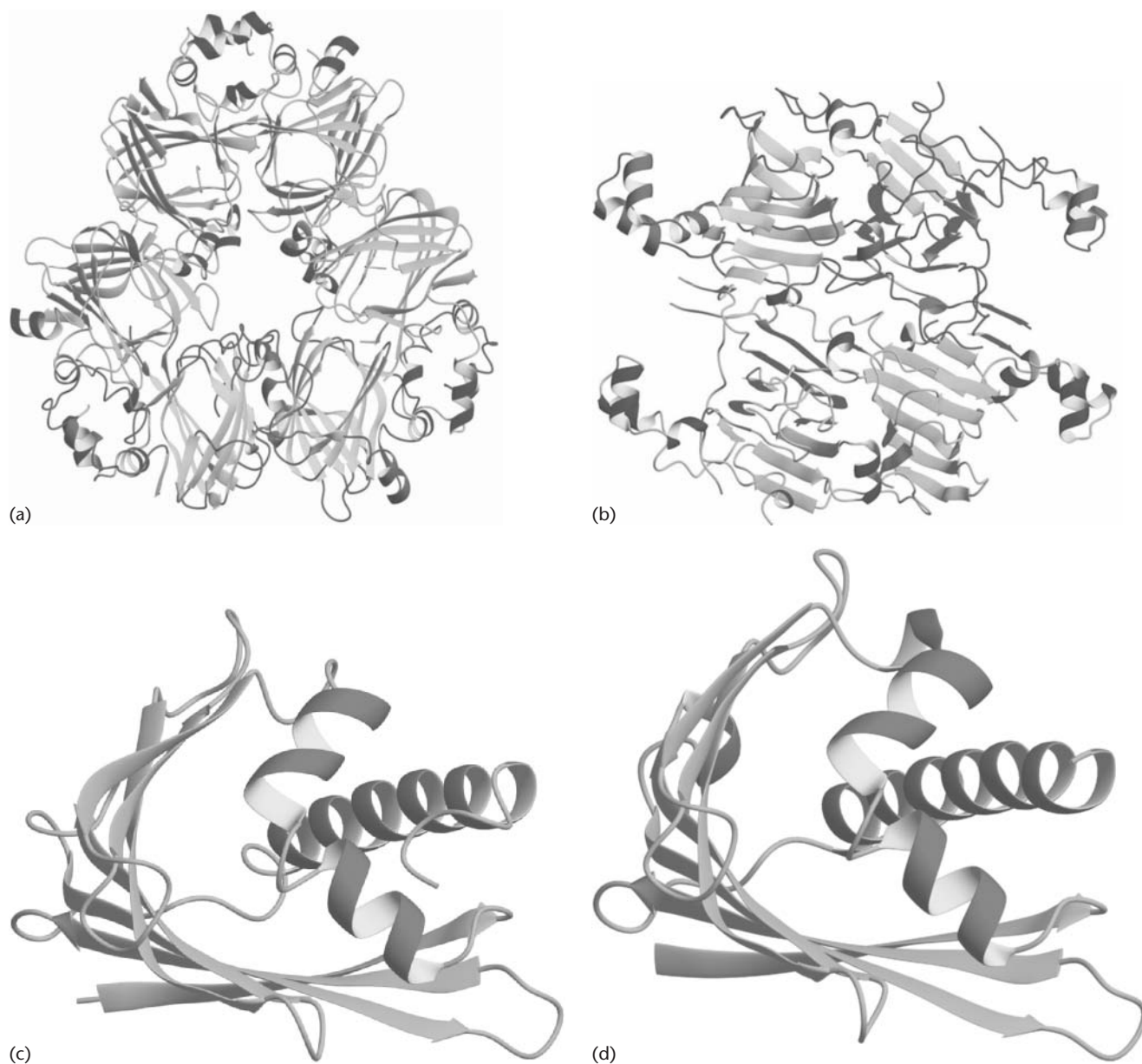


Figure 4.2 Typical structures of the cupin and Bet v 1 superfamily allergens shown as ribbon diagrams with ball-and-stick disulfides. PDB codes are given in parentheses. (a) Trimeric structure of soybean 7S globulin β -conglycinin comprising solely β -subunits (1UIJ). (b) Edge-on view of soybean 11S globulin showing the way in which individual subunits are stacked within the hexamer (2D5H). (c) Major birch pollen allergen Bet v 1 (1BV1). (d) Allergenic Bet v 1 homolog from celery root, Api g 1 (2BK0).

50,000. They are also termed vicilins since they are particularly found in the *Viciae* group of legumes. The subunits are again the products of a multigene family and also undergo proteolytic processing and glycosylation the extent of which varies depending on the plant species [145].

Major allergens include the 7S (Fig. 4.2(a)) and 11S globulins (Fig. 4.2(b)) of soybean [112,118,145], Ara h 1 and Ara h 3 of peanut [97,111,117], Ana c 1 and Ana c 2 of cashew nut [116,121], the 7S globulins Jug r 2 of walnut [114] and Len c 1 of lentil [147], and the 7S globulins of sesame [101] and hazelnut [144]. The 11S globulins have also been shown to be allergens in almond, also known as almond major protein (AMP) [148], and in hazelnut [149]. In general, these vicilin- and legumin-like seed globulins exhibit a high degree of thermostability, requiring temperatures in excess of 70°C for denaturation, and have a propensity to form large aggregates on heating which still retain, to a large degree, their native secondary structure [150–152]. At high protein concentrations these proteins can form heat-set gels [152].

Since the globulins are partially or fully insoluble between pH 3.5 and 6.5 it is likely that only limited solubilization of globulins would occur when they enter the stomach. However, the 7S globulins seem to be highly susceptible to pepsinolysis, although several LMW polypeptides seem to persist following digestion of the peanut 7S globulin allergen Ara h 1 [153,154], and there is evidence that they still possess IgE-binding sites following proteolysis [155]. Similarly *in vitro* simulated gastrointestinal digestion results in rapid and almost complete degradation of the protein to relatively small polypeptides although these retain their allergenic properties [156]. There are indications that the peptides do not remain monomeric but can assemble into larger structures and it may be that this propensity to aggregate is responsible for the protein retaining its allergenic properties even when hydrolyzed.

The Bet v 1 family

The association of plant food allergies with birch pollen allergy is the most frequently observed of the cross-reactivity syndromes [157]. The clinical symptoms of the birch pollen allergy-related OAS are caused by cross-reactive IgE between the major birch pollen allergen Bet v 1 (Fig. 4.2(c)) and its homologs in a wide range of fruits and vegetables, including apple [103], celery (Fig. 4.2(d)) [118], peanut [158], mung bean [159], sharon fruit [160], and even jackfruit [121]. Bet v 1 was the first allergen identified that possessed similarities to family 10 of the pathogenesis-related (PR) proteins [162,163]. As a possible biological function in plants, a general plant-steroid carrier function for Bet v 1 and related PR-10 proteins was suggested [164]. The known structures of Bet v 1 [164,165] and its homologs in cherry [166] and celery [167] illustrate the high identity of the molecular surfaces that are accessible to IgE and

thus offer a molecular explanation for the observed clinical cross-reactivities. A structural bioinformatic analysis of Bet v 1 and its homologous allergens from apple, soybean, and celery showed that conservation of three-dimensional structure plays an important role in conservation of IgE-binding epitopes and underlies the birch pollen – plant food syndrome [1]. There is evidence on the T-cell level that Bet v 1 is the relevant sensitizing agent [168]. It has been shown that a subpopulation of patients with birch pollen allergy and atopic dermatitis reacted with worsening of eczema after oral challenge with foods harboring Bet v 1 homologous proteins [169]. Bet v 1-specific T-cells could be found in the lesional skin of these patients [169]. T-cell cross-reactivity between Bet v 1 and related food allergens occurs independently of IgE cross-reactivity. Gastrointestinal or heat degradation destroyed the histamine releasing but not the T-cell activating properties of Bet v 1 homologous food allergens [170,171]. Thus, ingestion of cooked birch pollen allergy-related foods did not induce OAS but caused atopic eczema to worsen [171].

Minor families

Class I chitinases

Chitinases are enzymes that catalyze the hydrolysis of chitin polymers. Chitinases are members of the glycoside hydrolase families 18 or 19 [172]. Endochitinases from plants belong to 19 (also known as classes IA or I and IB or II) and are able to degrade chitin, a major structural component of the exoskeleton of insects and of the cell walls of many pathogenic fungi [173]. Class I chitinases contain an *N*-terminal so-called hevein domain with putative chitin-binding properties [174]. This hevein domain shares high sequence identity with the major *Hevea brasiliensis* latex allergen Hev b 6.02, hevein [175]. Class I chitinases from fruits such as avocado [176], banana [177], and chestnut [178] have been identified as major allergens that cross-react with Hev b 6.02. Pers a 1, an allergenic class I chitinase from avocado, was extensively degraded when subjected to simulated gastric fluid digestion. However, the resulting peptides, particularly those corresponding to the hevein-like domain, were clearly reactive both *in vitro* and *in vivo* [179]. The 43-residue polypeptide chain of hevein-like domains contains four disulfide bonds to which they owe their stability [180].

Cysteine protease superfamily

Cysteine proteases of the C1, or papain-like, family were originally characterized by having a cysteine residue as part of their catalytic site, which has now been extended to include conserved glutamine, cysteine, histidine, and asparagine residues [181]. Whilst sharing the fold of the C1 protease family, some members may have lost the capacity to act as proteases, a notable example being the soybean P34 protein in which a glycine has replaced the active site

cysteine residue [182]. Two major food allergens belong to this family, actinidin (Act c 1) from kiwi fruit [122] and an allergen involved in soybean-induced atopic dermatitis known as Gly m Bd 30K, Gly m 1, or P34 [123].

Profilins

Profilins from higher plants constitute a family of highly conserved proteins with sequence identities of at least 75% even between members of distantly related organisms [183]. Profilins are cytosolic proteins of 12–15 kDa in size that are found in all eukaryotic cells. Profilins bind to monomeric actin and a number of other proteins, thus regulating the actin polymerization and depolymerization during processes such as cell movement, cytokinesis, and signaling [184]. Originally, plant profilins were 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 [185]. Structures of three plant profilins have been elucidated so far, those from *Arabidopsis thaliana* pollen [186], birch pollen [187], and *Hevea brasiliensis* latex. Since profilin-specific IgE cross-reacts with homologs from virtually every plant source, sensitization to these allergens has been considered a risk factor for allergic reactions to multiple pollen sources [188] and for pollen-associated food allergy [189]. However, the clinical relevance of plant food profilin-specific IgE is still under debate [190]. Despite sequence identities of below 30%, plant profilin structures are highly similar to the structures of profilins from mammals, fungi, and amoeba. IgE directed to plant profilins weakly binds to the human homolog as well [191]. However, no profilins from sources other than plants have been shown to elicit allergic reactions.

Protease inhibitors and lectins

The Kunitz/bovine pancreatic trypsin inhibitor family is active against serine, thiol, aspartic, and subtilisin proteases. They are generally small (~50 residue) with three disulfide bonds constraining the proteins three-dimensional structure and belong to a superfamily of structurally related proteins, which share no sequence similarity and that includes such diverse proteins as interleukin-1 proteins, heparin-binding growth factors (HBGF), and histatophilin. In plants they probably play a role in defense against pests and pathogens. Minor allergens have been identified belonging to the Kunitz inhibitor family in soybean [192,193] and potato [194]. It is thought that their stability to processing and digestion is important for their allergenic activity. In addition to agglutinin, a lectin found in peanut has been identified as a minor allergen [193].

Thaumatococcal proteins

Thaumatococcal proteins (TLPs) derive their name from their sequence similarities to thaumatin, an intensely sweet tasting protein isolated from the fruits of the West African rain forest shrub *Thaumatococcus daniellii*. TLPs accumulate

in plants in response to pathogen challenge and belong to the PR-5 family of proteins that also includes thaumatin and osmotin [195]. Recent phylogenetic and structural studies revealed that PR-5 proteins constitute an ancient protein family that is conserved between plants, insects, and nematodes [6]. Several allergenic TLPs from fruits have been described. These include Mal d 2 from apple [196], Cap a 1 from bell pepper [197], Pru av 2 from sweet cherry [198,199], Act c 2 from kiwi [200], and an allergenic TLP from grape [77]. The conformation of TLPs is stabilized by eight disulfide bonds. This extensive disulfide cross-linking confers high stability to proteolysis to the TLP scaffold as has been shown for a zeamatin, a TLP from corn [201]. This is also the reason why the allergenic TLPs produced by grape berries persist during the entire vinification process and are among the major proteins present in wine [202].

What does this mean?

Many of the proteins that have been described as allergens in plant foods function as seed storage proteins, providing the nutrients for a developing plant, with the cow's milk caseins functioning in a similar fashion to provide essential nutrition to young mammals. This relationship may not be so surprising since these are the proteins predominantly found in nuts and seeds and as a consequence exposure in the human diet, especially to the abundant storage proteins, is considerable. Extent of exposure to a given protein probably plays a role in determining its allergenicity, with extensive exposure now thought to be important for tolerization, total exclusion precluding an individual from developing an allergy, with low levels of exposure possibly being more effective at sensitizing [203,204]. However, it is emerging that there is a complex dialog between different routes of exposure with evidence from animal models that cutaneous exposure may prevent oral tolerance developing [205].

There are a number of allergens which are less abundant in foods, notably the nsLTPs where the prolamin superfamily fold may play a role in potentiating the allergenicity of these proteins. Intriguingly many of the allergens that are less abundant in plant-derived foods have a role in plant protection. Others have alluded to the fact that many plant food allergens are involved in defense [206], with many of them being classified as pathogenesis-related proteins according to the criteria defined by van Loon and van Strien [207]. These include for example the PR-10 proteins from the Bet v 1 family of allergens, the PR-14 nsLTPs, whilst others, such as the cereal α -amylase inhibitors that have not been classified as PR proteins, are thought to have a protective function. In addition, certain minor animal food allergens also have an imputed protective function. Many PR proteins are resistant to the effects of extremes in pH and highly resistant to proteolysis, possibly to evade the degradative environment created by pests and pathogens infecting plant tissues [4].

It has been hypothesized that resistance to digestion is important in allowing sufficient immunologically active fragments to come into contact with the immune system, particularly with regards to sensitization via the gastrointestinal tract. However, it is evident that some allergen families, notably the caseins and the cupins, are readily degraded in the gastrointestinal tract. Nevertheless, for the cupins at least, there is evidence that degradation does not affect the ability of these proteins to elicit histamine release *in vitro* [156], although the impact of digestion on the sensitization potential of these proteins is not clear. For animal allergens there may also be the need to consider the evolutionary distance from man, since animal food allergens, notably the tropomyosins, lie at the borders of self-non-self recognition. Thus, it may be that in addition to the routes and extent of exposure, the mechanisms whereby different scaffolds sensitize and elicit allergic reactions may differ. Such complex interacting factors underlie the reasons why we still do not understand why some food proteins, and not others, cause allergic reactions in man. Other factors, such as the role of food processing and modification of allergens, or adjuvant effects of other food components, may also play a role in stimulating IgE, rather than IgG responses to foods such as peanuts. Only an improved understanding of these factors and the mechanisms underlying the generation of aberrant IgE responses will enable us to understand what makes a protein become an allergen.

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References

- Jenkins JA, Griffiths-Jones S, Shewry PR, *et al.* Structural relatedness of plant food allergens with specific reference to cross-reactive allergens: an *in silico* analysis. *J Allergy Clin Immunol* 2005;115:163–70.
- Radauer C, Breiteneder H. Pollen allergens are restricted to few protein families and show distinct patterns of species distribution. *J Allergy Clin Immunol* 2006;117:141–7.
- Dunwell JM, Purvis A, Khuri S. Cupins: the most functionally diverse protein superfamily? *Phytochemistry* 2004;65:7–17.
- 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;44:379–407.
- Rodin J, Rask L. Characterization of matheuccin, the 2.2S storage protein of the ostrich fern. Evolutionary relationship to angiosperm seed storage proteins. *Eur J Biochem* 1990;28:192:101–7.
- Shatters Jr RG, Boykin LM, Lapointe SL, *et al.* Phylogenetic and structural relationships of the PR5 gene family reveal an ancient multigene family conserved in plants and select animal taxa. *J Mol Evol* 2006;63:12–29.
- Liscombe DK, MacLeod BP, Loukanina N, *et al.* Evidence for the monophyletic evolution of benzyloisoquinoline alkaloid biosynthesis in angiosperms. *Phytochemistry* 2005;66:2501–20.
- Bateman A, Coin L, Durbin R, *et al.* The Pfam protein families database. *Nucleic Acids Res* 2004;1:32(Database issue):D138–41.
- Gendel SM, Jenkins JA. Allergen sequence databases. *Mol Nutr Food Res* 2006;50:633–7.
- Jenkins JA, Breiteneder H, Mills EN. Evolutionary distance from human homologs reflects allergenicity of animal food proteins. DOI information: 10.1016/j.jaci.2007.08.019.
- Breiteneder H, Radauer C. A classification of plant food allergens. *J Allergy Clin Immunol* 2004;113:821–30.
- Aalberse RC. Structural biology of allergens. *J Allergy Clin Immunol* 2000;106:228–38.
- MacLeod AR. Genetic origin of diversity of human cytoskeletal tropomyosins. *Bioessays* 1987;6:208–12.
- Li Y, Mui S, Brown JH, *et al.* The crystal structure of the C-terminal fragment of striated-muscle alpha-tropomyosin reveals a key troponin T recognition site. *Proc Natl Acad Sci USA* 2002;99:7378–83.
- Gunning PW, Schevzov G, Kee AJ, Hardeman EC. Tropomyosin isoforms: divining rods for actin cytoskeleton function. *Trends Cell Biol* 2005;15:333–41.
- Shanti KN, Martin BM, Nagpal S, *et al.* Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. *J Immunol* 1993;151:5354–63.
- Daul CB, Slattery M, Reese G, Lehrer SB. Identification of the major brown shrimp (*Penaeus aztecus*) allergen as the muscle protein tropomyosin. *Int Arch Allergy Immunol* 1994;105:49–55.
- Leung PS, Chu KH, Chow WK, *et al.* Cloning, expression, and primary structure of *Metapenaeus ensis* tropomyosin, the major heat-stable shrimp allergen. *J Allergy Clin Immunol* 1994;94:882–90.
- Reese G, Ayuso R, Lehrer SB. Tropomyosin: an invertebrate pan-allergen. *Int Arch Allergy Immunol* 1999;119:247–58.
- Motoyama K, Ishizaki S, Nagashima Y, Shiomi K. Cephalopod tropomyosins: identification as major allergens and molecular cloning. *Food Chem Toxicol* 2006;44:1997–2002.
- Naqpal S, Rajappa L, Metcalfe DD, Rao PV. Isolation and characterization of heat-stable allergens from shrimp (*Penaeus indicus*). *J Allergy Clin Immunol* 1989;83:26–36.
- Lehrer SB, Ibanez MD, McCants ML, *et al.* Characterization of water-soluble shrimp allergens released during boiling. *J Allergy Clin Immunol* 1990;85:1005–13.
- Lewit-Bentley A, Rety S. EF-hand calcium-binding proteins. *Curr Opin Struct Biol* 2000;10:637–43.
- Ikura M. Calcium binding and conformational response in EF-hand proteins. *Trends Biochem Sci* 1996;21:14–17.
- Declercq JP, Tinant B, Parello J, Rambaud J. Ionic interactions with parvalbumins. Crystal structure determination of pike

- 4.10 parvalbumin in four different ionic environments. *J Mol Biol* 1991;220:1017–39.
- 26 Pauls TL, Cox JA, Berchtold MW. The Ca²⁺(-)-binding proteins parvalbumin and oncomodulin and their genes: new structural and functional findings. *Biochim Biophys Acta* 1996;1306:39–54.
- 27 Bugajska-Schretter A, Elfman L, Fuchs T, *et al.* Parvalbumin, a cross-reactive fish allergen, contains IgE-binding epitopes sensitive to periodate treatment and Ca²⁺ depletion. *J Allergy Clin Immunol* 1998;101:67–74.
- 28 Bugajska-Schretter A, Grote M, Vangelista L, *et al.* Purification, biochemical, and immunological characterisation of a major food allergen: different immunoglobulin E recognition of the apo- and calcium-bound forms of carp parvalbumin. *Gut* 2000;46:661–9.
- 29 Filimonov VV, Pfeil W, Tsalkova TN, Privalov PL. Thermodynamic investigations of proteins. IV. Calcium binding protein parvalbumin. *Biophys Chem* 1978;8:117–22.
- 30 Elsayed S, Aas K. Characterization of a major allergen (cod). Observations on effect of denaturation on the allergenic activity. *J Allergy* 1971;47:283–91.
- 31 Aas K, Jebsen JW. Studies of hypersensitivity to fish. Partial purification and crystallization of a major allergenic component of cod. *Int Arch Allergy Appl Immunol* 1967;32:1–20.
- 32 Elsayed S, Bennich H. The primary structure of allergen M from cod. *Scand J Immunol* 1975;4:203–8.
- 33 Bernhisel-Broadbent J, Scanlon SM, Sampson HA. Fish hypersensitivity. I. *In vitro* and oral challenge results in fish-allergic patients. *J Allergy Clin Immunol* 1992;89:730–7.
- 34 Hilger C, Thill L, Grigioni F, *et al.* IgE antibodies of fish allergic patients cross-react with frog parvalbumin. *Allergy* 2004;59:653–60.
- 35 Hilger C, Grigioni F, Thill L, *et al.* Severe IgE-mediated anaphylaxis following consumption of fried frog legs: definition of alpha-parvalbumin as the allergen in cause. *Allergy* 2002;57:1053–8.
- 36 Tuinier R, de Kruif CG. Stability of casein micelles in milk. *J Chem Phys* 2002;117:1290–5.
- 37 Kawasaki K, Weiss KM. Mineralized tissue and vertebrate evolution: the secretory calcium-binding phosphoprotein gene cluster. *Proc Natl Acad Sci USA* 2003;100:4060–5.
- 38 Natale M, Bisson C, Monti G, *et al.* Cow's milk allergens identification by two-dimensional immunoblotting and mass spectrometry. *Mol Nutr Food Res* 2004;48:363–9.
- 39 Reese G, Tracey D, Daul CB, *et al.* IgE and monoclonal antibody reactivities to the major shrimp allergen Pen a 1 (tropomyosin) and vertebrate tropomyosins. *Adv Exp Med Biol* 1996;409:225–30.
- 40 Yu CJ, Lin YF, Chiang BL, Chow LP. Proteomics and immunological analysis of a novel shrimp allergen, Pen m 2. *J Immunol* 2003;170:445–53.
- 41 Asturias JA, Eraso E, Arilla MC, *et al.* Cloning, isolation, and IgE-binding properties of *Helix aspersa* (brown garden snail) tropomyosin. *Int Arch Allergy Immunol* 2002;128:90–6.
- 42 Miyazawa H, Fukamachi H, Inagaki Y, *et al.* Identification of the first major allergen of a squid (*Todarodes pacificus*). *J Allergy Clin Immunol* 1996;98:948–53.
- 43 Leung PS, Chu KH. cDNA cloning and molecular identification of the major oyster allergen from the Pacific oyster *Crassostrea gigas*. *Clin Exp Allergy* 2001;31:1287–94.
- 44 Leung PS, Chen YC, Gershwin ME, *et al.* Identification and molecular characterization of *Charybdis feriatus* tropomyosin, the major crab allergen. *J Allergy Clin Immunol* 1998;102:847–52.
- 45 Chu KH, Wong SH, Leung PS. Tropomyosin is the major mollusk allergen: reverse transcriptase polymerase chain reaction, Expression and IgE Reactivity. *Mar Biotechnol (NY)* 2000;2:499–509.
- 46 Van Do T, Hordvik I, Endresen C, *et al.* The major allergen (parvalbumin) of codfish is encoded by at least two isotypic genes: cDNA cloning, expression and antibody binding of the recombinant allergens. *Mol Immunol* 2003;39:595–602.
- 47 Swoboda I, Bugajska-Schretter A, Verdino P, *et al.* Recombinant carp parvalbumin, the major cross-reactive fish allergen: a tool for diagnosis and therapy of fish allergy. *J Immunol* 2002;168:4576–84.
- 48 Lindstrom CD, van Do T, Hordvik I, *et al.* Cloning of two distinct cDNAs encoding parvalbumin, the major allergen of Atlantic salmon (*Salmo salar*). *Scand J Immunol* 1996;44:335–44.
- 49 Shiomi K, Hamada Y, Sekiguchi K, *et al.* Two classes of allergens, parvalbumins and higher molecular weight substances, in Japanese eel and bigeye tuna. *Fish Sci* 1999;65:943–8.
- 50 Bernard H, Creminon C, Yvon M, *et al.* Specificity of the human IgE response to the different purified caseins in allergy to cow's milk proteins. *Int Arch Allergy Immunol* 1998;115:235–44.
- 51 Bellioni-Businco B, Paganelli R, Lucenti P, *et al.* Allergenicity of goat's milk in children with cow's milk allergy. *J Allergy Clin Immunol* 1999;103:1191–4.
- 52 Spuergerin P, Walter M, Schiltz E, *et al.* Allergenicity of alpha-caseins from cow, sheep, and goat. *Allergy* 1997;52:293–8.
- 53 Businco L, Giampietro PG, Lucenti P, *et al.* Allergenicity of mare's milk in children with cow's milk allergy. *J Allergy Clin Immunol* 2000;105:1031–4.
- 54 Restani P, Gaiaschi A, Plebani A, *et al.* Cross-reactivity between milk proteins from different animal species. *Clin Exp Allergy* 1999;29:997–1004.
- 55 Flower DR. The lipocalin protein family: structure and function. *Biochem J* 1996;318:1–14.
- 56 Virtanen T. Lipocalin allergens. *Allergy* 2001;56:48–51.
- 57 Nitta K, Sugai S. The evolution of lysozyme and alpha-lactalbumin. *Eur J Biochem* 1989;182:111–8.
- 58 Aabin B, Poulsen LK, Ebbehøj K, *et al.* Identification of IgE-binding egg white proteins: comparison of results obtained by different methods. *Int Arch Allergy Immunol* 1996;109:50–7.
- 59 Holen E, Elsayed S. Characterization of four major allergens of hen egg-white by IEF/SDS-PAGE combined with electrophoretic transfer and IgE-immunoautoradiography. *Int Arch Allergy Appl Immunol* 1990;91:136–41.
- 60 van Gent D, Sharp P, Morgan K, Kalsheker N. Serpins: structure, function and molecular evolution. *Int J Biochem Cell Biol* 2003;35:1536–47.
- 61 Bernhisel-Broadbent J, Dintzis HM, Dintzis RZ, Sampson HA. Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III)

- compared with ovalbumin (Gal d I) in children with egg allergy and in mice. *J Allergy Clin Immunol* 1994;93:1047–59.
- 62 Binder M, Mahler V, Hayek B, *et al.* Molecular and immunological characterization of arginine kinase from the Indianmeal moth, *Plodia interpunctella*, a novel cross-reactive invertebrate pan-allergen. *J Immunol* 2001;167:5470–7.
 - 63 Laskowski Jr M, Kato I, Ardelt W, *et al.* Ovomucoid third domains from 100 avian species: isolation, sequences, and hypervariability of enzyme-inhibitor contact residues. *Biochemistry* 1987;26:202–21.
 - 64 Kato I, Schrode J, Kohr WJ, Laskowski Jr. M. Chicken ovomucoid: determination of its amino acid sequence, determination of the trypsin reactive site, and preparation of all three of its domains. *Biochemistry* 1987;26:193–201.
 - 65 Cooke SK, Sampson HA. Allergenic properties of ovomucoid in man. *J Immunol* 1997;159:2026–32.
 - 66 Kreis M, Forde BG, Rahman S, *et al.* Molecular evolution of the seed storage proteins of barley, rye and wheat. *J Mol Biol* 1985;183:499–502.
 - 67 Palosuo K, Alenius H, Varjonen E, *et al.* A novel wheat gliadin as a cause of exercise-induced anaphylaxis. *J Allergy Clin Immunol* 1999;103:912–17.
 - 68 Palosuo K, Varjonen E, Kekki OM, *et al.* Wheat omega-5 gliadin is a major allergen in children with immediate allergy to ingested wheat. *J Allergy Clin Immunol* 2001;108:634–8.
 - 69 Maruyama N, Ichise K, Katsube T, *et al.* Identification of major wheat allergens by means of the *Escherichia coli* expression system. *Eur J Biochem* 1998;255:739–45.
 - 70 Sanchez-Monge R, Lombardero M, Garcia-Selles FJ, *et al.* Lipid-transfer proteins are relevant allergens in fruit allergy. *J Allergy Clin Immunol* 1999;103:514–9.
 - 71 Pastorello EA, D'Ambrosio FP, Pravettoni V, *et al.* Evidence for a lipid transfer protein as the major allergen of apricot. *J Allergy Clin Immunol* 2000;105:371–7.
 - 72 Scheurer S, Pastorello EA, Wangorsch A, *et al.* Recombinant allergens Pru av 1 and Pru av 4 and a newly identified lipid transfer protein in the *in vitro* diagnosis of cherry allergy. *J Allergy Clin Immunol* 2001;107:724–31.
 - 73 Pastorello EA, Farioli L, Pravettoni V, *et al.* The major allergen of peach (*Prunus persica*) is a lipid transfer protein. *J Allergy Clin Immunol* 1999;103:520–6.
 - 74 Pastorello EA, Farioli L, Pravettoni V, *et al.* Characterization of the major allergen of plum as a lipid transfer protein. *J Chromatogr B Biomed Sci Appl* 2001;756:95–103.
 - 75 Zuidmeer L, Salentijn E, Rivas MF, *et al.* The role of profilin and lipid transfer protein in strawberry allergy in the Mediterranean area. *Clin Exp Allergy* 2006;36:666–75.
 - 76 Ahrazem O, Ibanez MD, Lopez-Torrejón G, *et al.* Orange germin-like glycoprotein Cit s 1: an equivocal allergen. *Int Arch Allergy Immunol* 2006;139:96–103.
 - 77 Pastorello EA, Farioli L, Pravettoni V, *et al.* Identification of grape and wine allergens as an endochitinase 4, a lipid-transfer protein, and a thaumatin. *J Allergy Clin Immunol* 2003;111:350–9.
 - 78 Sanchez-Monge R, Blanco C, Lopez-Torrejón G, *et al.* Differential allergen sensitization patterns in chestnut allergy with or without associated latex-fruit syndrome. *J Allergy Clin Immunol* 2006;118:705–10.
 - 79 Asero R, Mistrello G, Roncarolo D, *et al.* Allergy to minor allergens of Brazil nut. *Allergy* 2002;57:1080–1.
 - 80 Pastorello EA, Farioli L, Pravettoni V, *et al.* Lipid transfer protein and vicilin are important walnut allergens in patients not allergic to pollen. *J Allergy Clin Immunol* 2004;114:908–14.
 - 81 Schocker F, Luttkopf D, Scheurer S, *et al.* Recombinant lipid transfer protein Cor a 8 from hazelnut: a new tool for *in vitro* diagnosis of potentially severe hazelnut allergy. *J Allergy Clin Immunol* 2004;113:141–7.
 - 82 Diaz-Perales A, Tabar AI, Sanchez-Monge R, *et al.* Characterization of asparagus allergens: a relevant role of lipid transfer proteins. *J Allergy Clin Immunol* 2002;110:790–6.
 - 83 San Miguel-Moncin M, Krail M, Scheurer S, *et al.* Lettuce anaphylaxis: identification of a lipid transfer protein as the major allergen. *Allergy* 2003;58:511–7.
 - 84 Palacin A, Cumplido J, Figueroa J, *et al.* Cabbage lipid transfer protein Bra o 3 is a major allergen responsible for cross-reactivity between plant foods and pollens. *J Allergy Clin Immunol* 2006;117:1423–9.
 - 85 Pastorello EA, Farioli L, Pravettoni V, *et al.* The maize major allergen, which is responsible for food-induced allergic reactions, is a lipid transfer protein. *J Allergy Clin Immunol* 2000;106:744–51.
 - 86 Curioni A, Santucci B, Cristaudo A, *et al.* Urticaria from beer: an immediate hypersensitivity reaction due to a 10-kDa protein derived from barley. *Clin Exp Allergy* 1999;29:407–13.
 - 87 Nakase M, Adachi T, Urisu A, *et al.* Rice (*Oryza sativa* L.) alpha amylase inhibitors of 14–16 kDa are potential allergens and products of a multi gene family. *J Agric Food Chem* 1996;44:2624–8.
 - 88 Alvarez AM, Adachi T, Nakase M, *et al.* Classification of rice allergenic protein cDNAs belonging to the alpha-amylase/trypsin inhibitor gene family. *Biochim Biophys Acta* 1995;1251:201–4.
 - 89 Izumi H, Adachi T, Fujii N, *et al.* Nucleotide sequence of a cDNA clone encoding a major allergenic protein in rice seeds. Homology of the deduced amino acid sequence with members of alpha-amylase/trypsin inhibitor family. *FEBS Lett* 1992;302:213–16.
 - 90 Teuber SS, Dandekar AM, Peterson WR, Sellers CL. Cloning and sequencing of a gene encoding a 2S albumin seed storage protein precursor from English walnut (*Juglans regia*), a major food allergen. *J Allergy Clin Immunol* 1998;101:807–14.
 - 91 Poltronieri P, Cappello MS, Dohmae N, *et al.* Identification and characterisation of the IgE-binding proteins 2S albumin and conglutin gamma in almond (*Prunus dulcis*) seeds. *Int Arch Allergy Immunol* 2002;128:97–104.
 - 92 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;102:1021–7.
 - 93 Robotham JM, Wang F, Seamon V, *et al.* Ana o 3, an important cashew nut (*Anacardium occidentale* L.) allergen of the 2S albumin family. *J Allergy Clin Immunol* 2005;115:1284–90.
 - 94 Menendez-Arias L, Moneo I, Dominguez J, Rodriguez R. Primary structure of the major allergen of yellow mustard (*Sinapis alba* L.) seed, Sin a I. *Eur J Biochem* 1988;177:159–66.

- 95 Monsalve RI, Gonzalez de la Pena MA, Menendez-Arias L, *et al.* Characterization of a new oriental-mustard (*Brassica juncea*) allergen, Bra j IE: detection of an allergenic epitope. *Biochem J* 1993;293:625–32.
- 96 Vioque J, Sanchez-Vioque R, Clemente A, *et al.* Purification and partial characterization of chickpea 2S albumin. *J Agric Food Chem* 1999;47:1405–9.
- 97 Burks AW, Williams LW, Connaughton C, *et al.* Identification and characterization of a second major peanut allergen, Ara h II, with use of the sera of patients with atopic dermatitis and positive peanut challenge. *J Allergy Clin Immunol* 1992;90:962–9.
- 98 Kleber-Janke T, Cramer R, Appenzeller U, *et al.* Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology. *Int Arch Allergy Immunol* 1999;119:265–74.
- 99 Stanley JS, King N, Burks AW, *et al.* Identification and mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen Ara h 2. *Arch Biochem Biophys* 1997;342:244–53.
- 100 Pastorello EA, Varin E, Farioli L, *et al.* The major allergen of sesame seeds (*Sesamum indicum*) is a 2S albumin. *J Chromatogr B Biomed Sci Appl* 2001;756:85–93.
- 101 Beyer K, Bardina L, Grishina G, Sampson HA. Identification of sesame seed allergens by 2-dimensional proteomics and Edman sequencing: seed storage proteins as common food allergens. *J Allergy Clin Immunol* 2002;110:154–9.
- 102 Kelly JD, Hlywka JJ, Hefle SL. Identification of sunflower seed IgE-binding proteins. *Int Arch Allergy Immunol* 2000;121:19–24.
- 103 Vanek-Krebitz M, Hoffmann-Sommergruber K, Laimer da Camara Machado M, *et al.* Cloning and sequencing of Mal d 1, the major allergen from apple (*Malus domestica*), and its immunological relationship to Bet v 1, the major birch pollen allergen. *Biochem Biophys Res Commun* 1995;214:538–51.
- 104 Karamloo F, Scheurer S, Wangorsch A, *et al.* Pyr c 1, the major allergen from pear (*Pyrus communis*), is a new member of the Bet v 1 allergen family. *J Chromatogr B Biomed Sci Appl* 2001;756:281–93.
- 105 Scheurer S, Metzner K, Haustein D, *et al.* Molecular cloning, expression and characterization of Pru a 1, the major cherry allergen. *Mol Immunol* 1997;34:619–29.
- 106 Karlsson AL, Alm R, Ekstrand B, *et al.* Bet v 1 homologues in strawberry identified as IgE-binding proteins and presumptive allergens. *Allergy* 2004;59:1277–84.
- 107 Hoffmann-Sommergruber K, O’Riordain G, Ahorn H, *et al.* Molecular characterization of Dau c 1, the Bet v 1 homologous protein from carrot and its cross-reactivity with Bet v 1 and Api g 1. *Clin Exp Allergy* 1999;29:840–7.
- 108 Breiteneder H, Hoffmann-Sommergruber K, O’Riordain G, *et al.* Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relationships to a group of 17-kDa tree pollen allergens. *Eur J Biochem* 1995;233:484–9.
- 109 Luttkopf D, Muller U, Skov PS, *et al.* Comparison of four variants of a major allergen in hazelnut (*Corylus avellana*) Cor a 1.04 with the major hazel pollen allergen Cor a 1.01. *Mol Immunol* 2002;38:515–25.
- 110 Mittag D, Vieths S, Vogel L, *et al.* Soybean allergy in patients allergic to birch pollen: clinical investigation and molecular characterization of allergens. *J Allergy Clin Immunol* 2004;113:148–54.
- 111 Burks AW, Williams LW, Helm RM, *et al.* Identification of a major peanut allergen, Ara h I, in patients with atopic dermatitis and positive peanut challenges. *J Allergy Clin Immunol* 1991;88:172–9.
- 112 Ogawa T, Bando N, Tsuji H, *et al.* Alpha-subunit of beta-conglycinin, an allergenic protein recognized by IgE antibodies of soybean-sensitive patients with atopic dermatitis. *Biosci Biotechnol Biochem* 1995;59:831–3.
- 113 Kondo Y, Urisu A, Wada E, *et al.* [Allergen analysis of buckwheat by the immunoblotting method]. *Arerugi* 1993;42:142–8.
- 114 Teuber SS, Jarvis KC, Dandekar AM, *et al.* Identification and cloning of a complementary DNA encoding a vicilin-like protein, jug r 2, from English walnut kernel (*Juglans regia*), a major food allergen. *J Allergy Clin Immunol* 1999;104:1311–20.
- 115 Lauer I, Foetisch K, Kolarich D, *et al.* Hazelnut (*Corylus avellana*) vicilin Cor a 11: molecular characterization of a glycoprotein and its allergenic activity. *Biochem J* 2004;383:327–34.
- 116 Wang F, Robotham JM, Teuber SS, *et al.* Ana o 1, a cashew (*Anacardium occidentale*) allergen of the vicilin seed storage protein family. *J Allergy Clin Immunol* 2002;110:160–6.
- 117 Rabjohn P, Helm EM, Stanley JS, *et al.* Molecular cloning and epitope analysis of the peanut allergen Ara h 3. *J Clin Invest* 1999;103:535–42.
- 118 Beardslee TA, Zeece MG, Sarath G, *et al.* Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen Ara h 3. *Int Arch Allergy Immunol* 2000;123:299–307.
- 119 Garcia-Mas J, Messeguer R, Arus P, *et al.* Molecular characterization of cDNAs corresponding to genes expressed during almond (*Prunus amygdalus Batsch*) seed development. *Plant Mol Biol* 1995;27:205–10.
- 120 Roux KH, Teuber SS, Robotham JM, *et al.* Detection and stability of the major almond allergen in foods. *J Agric Food Chem* 2001;49:2131–6.
- 121 Wang F, Robotham JM, Teuber SS, *et al.* Ana o 2, a major cashew (*Anacardium occidentale* L.) nut allergen of the legumin family. *Int Arch Allergy Immunol* 2003;132:27–39.
- 122 Pastorello EA, Conti A, Pravettoni V, *et al.* Identification of actinidin as the major allergen of kiwi fruit. *J Allergy Clin Immunol* 1998;101:531–7.
- 123 Ogawa T, Tsuji H, Bando N, *et al.* Identification of the soybean allergenic protein, Gly m Bd 30K, with the soybean seed 34-kDa oil-body-associated protein. *Biosci Biotechnol Biochem* 1993;57:1030–3.
- 124 Tassin-Moindrot S, Caille A, Douliez JP, *et al.* The wide binding properties of a wheat nonspecific lipid transfer protein. Solution structure of a complex with prostaglandin B2. *Eur J Biochem* 2000;267:1117–24.
- 125 Douliez JP, Jegou S, Pato C, *et al.* Binding of two monoacylated lipid monomers by the barley lipid transfer protein,

- LTP1, as viewed by fluorescence, isothermal titration calorimetry and molecular modelling. *Eur J Biochem* 2001;268:384–8.
- 126 Pastorello EA, Pompei C, Pravettoni V, *et al.* Lipid-transfer protein is the major maize allergen maintaining IgE-binding activity after cooking at 100 degrees C, as demonstrated in anaphylactic patients and patients with positive double-blind, placebo-controlled food challenge results. *J Allergy Clin Immunol* 2003;112:775–83.
 - 127 Sancho AI, Rigby NM, Zuidmeer L, *et al.* The effect of thermal processing on the IgE reactivity of the non-specific lipid transfer protein from apple, Mal d 3. *Allergy* 2005;60:1262–8.
 - 128 Moreno FJ, Mellon FA, Wickham MS, *et al.* Stability of the major allergen Brazil nut 2S albumin (Ber e 1) to physiologically relevant *in vitro* gastrointestinal digestion. *FEBS J* 2005;272:341–52.
 - 129 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;122:20–32.
 - 130 Vassilopoulou E, Rigby N, Moreno FJ, *et al.* Effect of *in vitro* gastric and duodenal digestion on the allergenicity of grape lipid transfer protein. *J Allergy Clin Immunol* 2006;118:473–80.
 - 131 Shewry PR, Halford NG, Belton PS, Tatham AS. The structure and properties of gluten: an elastic protein from wheat grain. *Philos Trans Roy Soc Lond B Biol Sci* 2002;357:133–42.
 - 132 Matsuo H, Morita E, Tatham AS, *et al.* Identification of the IgE-binding epitope in omega-5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. *J Biol Chem* 2004;279:12135–40.
 - 133 Watanabe M, Tanabe S, Suzuki T, *et al.* Primary structure of an allergenic peptide occurring in the chymotryptic hydrolysate of gluten. *Biosci Biotechnol Biochem* 1995;59:1596–7.
 - 134 Tanabe S, Arai S, Yanagihara Y, *et al.* A major wheat allergen has a Gln-Gln-Gln-Pro-Pro motif identified as an IgE-binding epitope. *Biochem Biophys Res Commun* 1996;219:290–3.
 - 135 Simonato B, Pasini G, Giannattasio M, *et al.* Food allergy to wheat products: the effect of bread baking and *in vitro* digestion on wheat allergenic proteins. A study with bread dough, crumb, and crust. *J Agric Food Chem* 2001;49:5668–73.
 - 136 Simonato B, De Lazzari F, Pasini G, *et al.* IgE binding to soluble and insoluble wheat flour proteins in atopic and non-atopic patients suffering from gastrointestinal symptoms after wheat ingestion. *Clin Exp Allergy* 2001;31:1771–8.
 - 137 Carbonero P, García-Olmedo F. *A Multigene Family of Trypsin/Alpha-Amylase Inhibitors from Cereals*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 1999.
 - 138 James JM, Sixbey JP, Helm RM, *et al.* Wheat alpha-amylase inhibitor: a second route of allergic sensitization. *J Allergy Clin Immunol* 1997;99:239–44.
 - 139 Shewry PR, Pandya MJ. *The 2S Albumin Storage Proteins*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 1999.
 - 140 Shibasaki M, Suzuki S, Tajima S, *et al.* Allergenicity of major component proteins of soybean. *Int Arch Allergy Appl Immunol* 1980;61:441–8.
 - 141 Duro G, Colombo P, Costa MA, *et al.* cDNA cloning, sequence analysis and allergological characterization of Pari j 2.0101, a new major allergen of the *Parietaria judaica* pollen. *FEBS Lett* 1996;399:295–8.
 - 142 Tejera ML, Villalba M, Batanero E, Rodriguez R. Identification, isolation, and characterization of Ole e 7, a new allergen of olive tree pollen. *J Allergy Clin Immunol* 1999;104:797–802.
 - 143 Pastorello EA, Farioli L, Robino AM, *et al.* A lipid transfer protein involved in occupational sensitization to spelt. *J Allergy Clin Immunol* 2001;108:145–6.
 - 144 Pastorello EA, Vieths S, Pravettoni V, *et al.* Identification of hazelnut major allergens in sensitive patients with positive double-blind, placebo-controlled food challenge results. *J Allergy Clin Immunol* 2002;109:563–70.
 - 145 Mills ENC, Jenkins JA, Bannon GA. *Plant Seed Globulin Allergens*. Oxford: Blackwell Publishing, 2004.
 - 146 Burks Jr AW, Brooks JR, Sampson HA. Allergenicity of major component proteins of soybean determined by enzyme-linked immunosorbent assay (ELISA) and immunoblotting in children with atopic dermatitis and positive soy challenges. *J Allergy Clin Immunol* 1988;81:1135–42.
 - 147 Lopez-Torreson G, Salcedo G, Martin-Esteban M, *et al.* Len c 1, a major allergen and vicilin from lentil seeds: protein isolation and cDNA cloning. *J Allergy Clin Immunol* 2003;112:1208–15.
 - 148 Roux KH, Teuber SS, Sathe SK. Tree nut allergens. *Int Arch Allergy Immunol* 2003;131:234–44.
 - 149 Beyer K, Grishina G, Bardina L, *et al.* Identification of an 11S globulin as a major hazelnut food allergen in hazelnut-induced systemic reactions. *J Allergy Clin Immunol* 2002;110:517–23.
 - 150 Mills EN, Huang L, Noel TR, *et al.* Formation of thermally induced aggregates of the soya globulin beta-conglycinin. *Biochim Biophys Acta* 2001;1547:339–50.
 - 151 Mills EN, Marigheto NA, Wellner N, *et al.* Thermally induced structural changes in glycinin, the 11S globulin of soya bean (*Glycine max*) – an *in situ* spectroscopic study. *Biochim Biophys Acta* 2003;1648:105–14.
 - 152 Yamauchi F, Yamagishi T, Iwabuchi S. Molecular understanding of heat-induced phenomena of soybean protein. *Food Rev Int* 1991;7:283–322.
 - 153 Maleki SJ, Kopper RA, Shin DS, *et al.* Structure of the major peanut allergen Ara h 1 may protect IgE-binding epitopes from degradation. *J Immunol* 2000;164:5844–9.
 - 154 Kopper RA, Odum NJ, Sen M, *et al.* Peanut protein allergens: gastric digestion is carried out exclusively by pepsin. *J Allergy Clin Immunol* 2004;114:614–8.
 - 155 Shin DS, Compadre CM, Maleki SJ, *et al.* Biochemical and structural analysis of the IgE binding sites on ara h1, an abundant and highly allergenic peanut protein. *J Biol Chem* 1998;273:13753–9.
 - 156 Eiwegger T, Rigby N, Mondoulet L, *et al.* Gastro-duodenal digestion products of the major peanut allergen Ara h 1 retain an allergenic potential. *Clin Exp Allergy* 2006;36:1281–8.
 - 157 Vieths S, Scheurer S, Ballmer-Weber B. Current understanding of cross-reactivity of food allergens and pollen. *Ann NY Acad Sci* 2002;964:47–68.

- 158 Mittag D, Akkerdaas J, Ballmer-Weber BK, *et al.* Ara h 8, a Bet v 1-homologous allergen from peanut, is a major allergen in patients with combined birch pollen and peanut allergy. *J Allergy Clin Immunol* 2004;114:1410–17.
- 159 Mittag D, Vieths S, Vogel L, *et al.* Birch pollen-related food allergy to legumes: identification and characterization of the Bet v 1 homologue in mungbean (*Vigna radiata*), Vig r 1. *Clin Exp Allergy* 2005;35:1049–55.
- 160 Bolhaar ST, van Ree R, Ma Y, *et al.* Severe allergy to sharon fruit caused by birch pollen. *Int Arch Allergy Immunol* 2005;136:45–52.
- 161 Bolhaar ST, Ree R, Bruijnzeel-Koomen CA, *et al.* Allergy to jackfruit: a novel example of Bet v 1-related food allergy. *Allergy* 2004;59:1187–92.
- 162 Breiteneder H, Pettenburger K, Bito A, *et al.* The gene coding for the major birch pollen allergen Betv1, is highly homologous to a pea disease resistance response gene. *EMBO J* 1989;8:1935–8.
- 163 Hoffmann-Sommergruber K. Plant allergens and pathogenesis-related proteins. What do they have in common? *Int Arch Allergy Immunol* 2000;122:155–66.
- 164 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;325:123–33.
- 165 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;3:1040–5.
- 166 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;276:22756–63.
- 167 Schirmer T, Hoffmann-Sommergrube K, Susani M, *et al.* Crystal structure of the major celery allergen Api g 1: molecular analysis of cross-reactivity. *J Mol Biol* 2005;351:1101–9.
- 168 Bohle B, Radakovics A, Jahn-Schmid B, *et al.* Bet v 1, the major birch pollen allergen, initiates sensitization to Api g 1, the major allergen in celery: evidence at the T cell level. *Eur J Immunol* 2003;33:3303–10.
- 169 Reekers R, Busche M, Wittmann M, *et al.* Birch pollen-related foods trigger atopic dermatitis in patients with specific cutaneous T-cell responses to birch pollen antigens. *J Allergy Clin Immunol* 1999;104:466–72.
- 170 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;116:1327–33.
- 171 Bohle B, Zwolfer B, Heratizadeh A, *et al.* Cooking birch pollen-related food: divergent consequences for IgE- and T cell-mediated reactivity *in vitro* and *in vivo*. *J Allergy Clin Immunol* 2006;118:242–9.
- 172 Henrissat B. A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem J* 1991;280:309–16.
- 173 Kasprzewska A. Plant chitinases – regulation and function. *Cell Mol Biol Lett* 2003;8:809–24.
- 174 Beintema JJ. Structural features of plant chitinases and chitin-binding proteins. *FEBS Lett* 1994;350:159–63.
- 175 Salcedo G, Diaz-Perales A, Sanchez-Monge R. The role of plant panallergens in sensitization to natural rubber latex. *Curr Opin Allergy Clin Immunol* 2001;1:177–83.
- 176 Sowka S, Hsieh LS, Krebitz M, *et al.* Identification and cloning of prs a 1, a 32-kDa endochitinase and major allergen of avocado, and its expression in the yeast *Pichia pastoris*. *J Biol Chem* 1998;273:28091–7.
- 177 Sanchez-Monge R, Blanco C, Diaz-Perales A, *et al.* Isolation and characterization of major banana allergens: identification as fruit class I chitinases. *Clin Exp Allergy* 1999;29:673–80.
- 178 Diaz-Perales A, Collada C, Blanco C, *et al.* Class I chitinases with hevein-like domain, but not class II enzymes, are relevant chestnut and avocado allergens. *J Allergy Clin Immunol* 1998;102:127–33.
- 179 Diaz-Perales A, Blanco C, Sanchez-Monge R, *et al.* Analysis of avocado allergen (Prs a 1) IgE-binding peptides generated by simulated gastric fluid digestion. *J Allergy Clin Immunol* 2003;112:1002–7.
- 180 Breiteneder H, Mills EN. Molecular properties of food allergens. *J Allergy Clin Immunol* 2005;115:14–23; quiz 4.
- 181 Rawlings ND, Barrett AJ. Evolutionary families of peptidases. *Biochem J* 1993;290:205–18.
- 182 Kalinski A, Weisemann JM, Matthews BF, *et al.* Molecular cloning of a protein associated with soybean seed oil bodies that is similar to thiol proteases of the papain family. *J Biol Chem* 1990;265:13843–8.
- 183 Radauer C, Willeroider M, Fuchs H, *et al.* Cross-reactive and species-specific immunoglobulin E epitopes of plant profilins: an experimental and structure-based analysis. *Clin Exp Allergy* 2006;36:920–9.
- 184 Witke W. The role of profilin complexes in cell motility and other cellular processes. *Trends Cell Biol* 2004;14:461–9.
- 185 Radauer C, Hoffmann-Sommergrube K. *Profilins*. Oxford: Blackwell Publishing, 2004.
- 186 Thorn KS, Christensen HE, Shigeta R, *et al.* The crystal structure of a major allergen from plants. *Structure* 1997; 5:19–32.
- 187 Fedorov AA, Ball T, Mahoney NM, *et al.* The molecular basis for allergen cross-reactivity: crystal structure and IgE-epitope mapping of birch pollen profilin. *Structure* 1997;5:33–45.
- 188 Mari A. Multiple pollen sensitization: a molecular approach to the diagnosis. *Int Arch Allergy Immunol* 2001;125:57–65.
- 189 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;112:427–32.
- 190 Wensing M, Akkerdaas JH, van Leeuwen WA, *et al.* IgE to Bet v 1 and profilin: cross-reactivity patterns and clinical relevance. *J Allergy Clin Immunol* 2002;110:435–42.
- 191 Valenta R, Duchene M, Pettenburger K, *et al.* Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* 1991;253:557–60.
- 192 Moroz LA, Yang WH. Kunitz soybean trypsin inhibitor: a specific allergen in food anaphylaxis. *N Engl J Med* 1980;302: 1126–8.

- 193 Burks AW, Cockrell G, Connaughton C, *et al.* Identification of peanut agglutinin and soybean trypsin inhibitor as minor legume allergens. *Int Arch Allergy Immunol* 1994;105:143–9.
- 194 Seppala U, Majamaa H, Turjanmaa K, *et al.* Identification of four novel potato (*Solanum tuberosum*) allergens belonging to the family of soybean trypsin inhibitors. *Allergy* 2001;56:619–26.
- 195 van Loon LC, Rep M, Pieterse CM. Significance of inducible defense-related proteins in infected plants. *Annu Rev Phytopathol* 2006;44:135–62.
- 196 Krebitz M, Wagner B, Ferreira F, *et al.* Plant-based heterologous expression of Mal d 2, a thaumatin-like protein and allergen of apple (*Malus domestica*), and its characterization as an antifungal protein. *J Mol Biol* 2003;329:721–30.
- 197 Fuchs HC, Hoffmann-Sommergrube K, Wagner B, *et al.* Heterologous expression in *Nicotiana benthamiana* of Cap a 1, a thaumatin-like protein and major allergen from bell pepper (*Capsicum annuum*). *J Allergy Clin Immunol* 2002;109(S):134–5.
- 198 Inschlag C, Hoffmann-Sommergruber K, O’Riordain G, *et al.* Biochemical characterization of Pru a 2, a 23-kD thaumatin-like protein representing a potential major allergen in cherry (*Prunus avium*). *Int Arch Allergy Immunol* 1998;116:22–8.
- 199 Fuchs HC, Bohle B, Dall’Antonia Y, *et al.* Natural and recombinant molecules of the cherry allergen Pru av 2 show diverse structural and B cell characteristics but similar T cell reactivity. *Clin Exp Allergy* 2006;36:359–68.
- 200 Gavrovic-Jankulovic M, cirkovic T, Vuckovic O, *et al.* Isolation and biochemical characterization of a thaumatin-like kiwi allergen. *J Allergy Clin Immunol* 2002;110:805–10.
- 201 Roberts WK, Selitrennikoff CP. Zeamatin, an antifungal protein from maize with membrane-permeabilizing activity. *J Gen Microbiol* 1990;136:1771–8.
- 202 Flamini R, De Rosso M. Mass spectrometry in the analysis of grape and wine proteins. *Expert Rev Proteomics* 2006;3:321–31.
- 203 Lack G, Golding J. Peanut and nut allergy. Reduced exposure might increase allergic sensitisation. *BMJ* 1996;313:300.
- 204 Strid J, Thomson M, Hourihane J, *et al.* A novel model of sensitization and oral tolerance to peanut protein. *Immunology* 2004;113:293–303.
- 205 Strid J, Hourihane J, Kimber I, *et al.* Epicutaneous exposure to peanut protein prevents oral tolerance and enhances allergic sensitization. *Clin Exp Allergy* 2005;35:757–66.
- 206 Hoffmann-Sommergruber K. Pathogenesis-related (PR)-proteins identified as allergens. *Biochem Soc Trans* 2002;30:930–5.
- 207 Van Loon LC, Van Strien EA. The families of pathogenesis related proteins, their activities, and comparative analysis of PR-1-type proteins. *Physiol Mol Plant Pathol* 1999;5:85–97.