

Overview of Plant Chitinases Identified as Food Allergens

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ABSTRACT: Food allergies are induced by proteins belonging to a limited number of families. Unfortunately, relationships between protein structure and capacity to induce the immune response have not been completely clarified yet, which precludes possible improvements in the diagnosis, prevention, and therapy of allergies. Plant chitinases constitute a good example of food allergenic proteins for which structural analysis of allergenicity has only been carried out partially. In plants, there are at least five structural classes of chitinases plus a number of chitinase-related polypeptides. Their allergenicity has been mostly investigated for chitinases of class I, due to both their higher prevalence among plant chitinases and by the high structural similarity between their substrate-binding domain and hevein, a well-known allergen present in the latex of rubber trees. Even if allergenic molecules have been identified for at least three other classes of plant chitinases, the involvement of the different structural motifs in the allergenicity of molecules has been disregarded so far. In this review, we provide a structurally based catalog of plant chitinases investigated for allergenicity, which could be a useful base for further studies aimed at better clarifying the structure–allergenicity relationships for this protein family.

KEYWORDS: plant food, food allergy, chitinase, allergen

■ INTRODUCTION

Food allergies are recognized as a major health concern, affecting about 4% of the total world population, and reaching peaks of 8% in infants.¹ In recent years, their occurrence in Westernized countries has shown a steady increasing trend, and currently no viable treatments are available for people with food allergies.² Food allergies are determined by a limited number of proteins, which, recognized as allergens by specific IgE, cause the release of histamine from mastocytes and basophils with resulting symptoms of variable severity, from mild pruritis to life-threatening anaphylaxis.

Among the more than 13,000 protein families listed in the Pfam database (<http://pfam.sanger.ac.uk/>),³ food allergens fall within a relatively small group of about 30 families,^{4–6} which are also described in Allfam (<http://www.meduniwien.ac.at/allergens/allfam/>), a database specific for allergen families.⁴ Biological activity of these proteins is even more restricted being limited to few biochemical functions: hydrolysis of proteins, polysaccharides, and lipids; binding of metal ions and lipids; and storage of nutrients and cytoskeleton association.⁴ These findings led to the assumption that allergens must meet specific, but not yet completely elucidated, structural features which are at the basis of their allergenic potency. Their disclosure could greatly accelerate research advances in the prevention, diagnosis, and therapy of allergies. Additionally, in the case of food allergens, uncovering the structural basis of allergenicity would provide insights for understanding the effects of food processing and contribute to a safer management of food proteins.⁷ There are examples of food allergens which retain allergenic potency even after cooking or transformation processes as has been shown for Cor a 1 in roasted hazelnuts⁸ and nonspecific lipid transfer proteins (nsLTP) in some plant

derived foods (sterilized peach juice and polenta)⁹ and fermented products (wine and beer).^{10,11} While strong structural constraints (such as disulfide bridges) can be evoked to explain the maintaining of some conformational epitopes and allergenicity for cooked food proteins, the allergenic potency that unfolded proteins still maintain can be attributed to their linear epitopes, as in the case of “fermented” nsLTPs and caseins (Mills et al.⁷ and references therein). In any case, at the moment there are no definitive, reliable ways to predict structural signatures for allergens in general and food allergens in particular.⁴

Food allergens can be distinguished as those that are directly involved in the sensitization process in the gastrointestinal tract, which generally show high stability toward heating and proteolysis, and those that cross-react with IgE related to pollen or other nonfood allergens, which are mostly easily degradable.¹² Cross-reactivity syndromes, i.e., the onset of food allergies in subjects already sensitized to nonfood allergens, are usually determined by similar structural features among allergens.¹³

Among food allergens, those of plant origin constitute an essential part, and numerous reviews are available reporting their classification and discussions about biological function, cross-reactivity syndromes, influence of protein structure on allergenic potency, and effects of cooking and other food processing as well.^{1,6,7,13,14} Here, we report a comprehensive analysis on the involvement of plant chitinases in food-allergies.

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Chitinases are enzymes that catalyze the hydrolysis of chitin, a linear homopolymer of β -1,4-*N*-acetyl-D-glucosamine units. They are ubiquitous in nature, having been detected in animals, plants, fungi, bacteria, and viruses. In plants, chitinases play a major role as pathogenesis related (PR) proteins, active in defense mechanisms against fungi and pathogens.^{15,16} While a clear structural classification of plant chitinases has long been reported,^{17–19} a precise classification of plant chitinases as allergens is still missing. The aim of this review is to update the knowledge on the relationships between the structures and properties of plant chitinases and their allergenicity. It will furnish the basis to establish in the future more detailed analyses of the structural determinants of allergenic chitinases.

■ CLASSIFICATION OF PLANT CHITINASES

Chitinases are classified into families 18 or 19 of glycoside hydrolases (GH), depending on the adoption of the so-called *retaining* or *inverting* hydrolysis mechanism, which results in the retention or inversion of the anomeric configuration of the hydrolysis product, respectively.²⁰ While chitinases of family 18 have heterogeneous origin, chitinases of family 19 mostly belong to plant and bacterial species.

From a structural point of view, plant chitinases of either families 18 or 19 can be further classified according to the presence of specific modules. Indeed, in addition to essential presequence (PM) and catalytic (CM) modules, the presence of additional chitin-binding (CtBM) and linker (LM) modules distinguishes particular classes. CMs can also exhibit different lengths, similarities, and architectures, according to the presence of structural loops on the protein surfaces. A schematic representation of the identified structural classes is reported in Figure 1. Only classes I and IV have the CtBM and

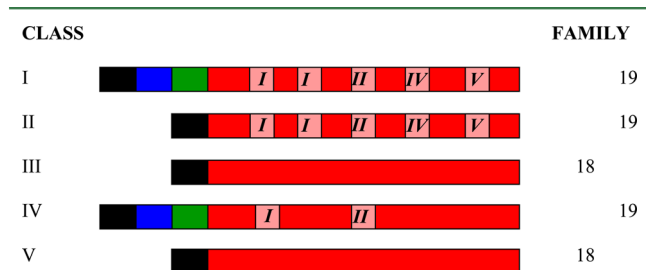


Figure 1. Schematic representation of different classes of chitinases of the 18 and 19 GH families. Color code: black = PM, blue = CtBM, green = LM, red = CM, and pink = surface loops. In order to adhere to previous literature, classes and surface loops have been both indicated with Roman figures, but italic font has been introduced for loops. Lengths of structural modules and positions of surface loops are only indicative. Adapted with permission from ref 24. Copyright 2009 Springer.

LM modules. CM modules can be distinguished on the basis of sequence similarities with reference enzymes (see Levorson and Chlan²¹ and references therein). Class I and II chitinases, which share highly homologous CM modules, are at least 50% identical to tobacco Chial chitinases.¹⁸ Chitinases of class III show no sequence similarities with class I or II chitinases and have higher than 50% sequence identities with a tobacco class III/lysozyme enzyme.²² Class IV chitinases have CM modules which are at least 50% identical to the *Phaseolus vulgaris* PR-4 chitinase. CM modules in class IV chitinases are about 50 amino acids shorter than in class I and II chitinases. In chitinases of the classes I, II, and IV, up to five surface loops can

be identified in the CM modules.^{23,24} Chitinases of class V have CM modules which share consensus motifs with those of class III but are clearly distinguishable on the basis of an additional α/β domain (see the Class V section).

■ CLASSES OF PLANT CHITINASES AND ALLERGIES

The possibility that plant chitinases may act as food allergens¹³ constitutes an important problem for human nutrition, also due to specific features of these proteins, which can be either overexpressed as PR-proteins^{25–28} or can be unexpectedly present as transgenic chitinases, used for enhancing resistance to fungi and pathogens^{29–31} (see the Class I section for a discussion about the induced expression of allergenic chitinases).

It has long been accepted that the allergenicity of plant chitinases derives from the presence of the CtBM, according to its high identity (65–70%) with hevein, a major allergen identified in the latex of rubber trees (*Hevea brasiliensis*).³² Therefore, class I chitinases were the main object of the first studies on allergenic chitinases. It must be clarified that hevein, even if essentially constituted by a CtBM, is not a chitinase (its characteristics are further described in the Other Chitinase-Related Allergens section). Because of the presence of common or highly similar structural epitopes, hevein and class I chitinases have been indicated as largely responsible for the cross-reactive syndrome known as latex-fruit syndrome.^{33,34} However, even at lower levels than hevein other latex allergens can be involved in latex-fruit cross-reactivity (see Radauer et al.³⁵ and references therein). Avocado, banana, chestnut, and kiwi fruits are considered as those with the highest degree of association to latex allergy.

Only more recently have chitinases from other classes been identified as allergens, resulting in a larger array of fruits and vegetables containing possible chitinase-type allergens. A summary of plant chitinases and structurally related proteins (hevein and other CtBM-like polypeptides), which have been indicated as allergens on the basis of their IgE-binding capacity, is reported in Table 1. With the exception of specific allergens detected in rubber tree latex, Japanese cedar pollen, and obeche dust, all the allergens can be regarded as food (or food-related) allergens. A literature-based list of allergenic chitinases is available in the Allergome database (<http://www.allergome.org/>). It must be emphasized that different approaches have been adopted to study the allergenicity of these chitinases (and of all allergens in general) and that not all are equivalent. For some of them, a rigorous analysis has been carried out, using both purified and recombinant proteins to study the alleged allergenicity. In a number of cases, the polypeptide has only been identified on the basis of partial sequence or mass-spectrometry analysis of molecules detected by either immunodetection assays using specific antibodies or immunoblot experiments carried out with sera of allergic patients. However, their isolation and complete characterization have not yet been accomplished. Up to now, chitinases acting as possible allergens have been identified in classes I–IV of plant chitinases. Details of studies carried out to investigate the allergenic activity of chitinases in each of the different structural classes, together with a short description of the main structural features, are given below.

Class I. This class contains most of the chitinases identified as allergens in plants. The first plant fruit chitinase with the capacity to bind IgE from allergic subjects was described in avocado (*Persea americana*) and is known by the name Pers a

Table 1. Classification of Plant Allergenic Chitinases^a

plant	accession number	chitinase class	allergene (IUIS database) ^b	structure	ref
<i>Actidinia chinensis</i> (gold kiwi)	c	I			67, 68
<i>Brassica rapa</i> (turnip)	P81 729	I	Bra r 2		27
<i>Carica papaya</i> (papaya)	d				69
<i>Castanea sativa</i> (chestnut)	Q42 428	I	Cas s 5		38, 70
<i>Hevea brasiliensis</i> (rubber tree)	Q8GUD7	I	Hev b 11		39, 53
<i>Lycopersicum esculentum</i> (tomato)	Q05538	I			40
<i>Musa</i> sp. (banana)	B6UYK6, C3VD22, M0SI55, M0SI56, 022318, Q8VXF1	I	Mus a 2		37, 71, 72
<i>Persea americana</i> (avocado)	P93 680	I	Pers a 1		36, 38, 70, 73
<i>Phaseolus vulgaris</i> (green bean)	P36 361	I	e, f		26
<i>Triplochiton scleroxylon</i> (obeche)		I	Trip s 1 e(wood dust)		74
<i>Triticum aestivum</i> (wheat)	Q6T484	I	Tri a endochitinase ^{g,e,h}		28
<i>Lycopersicum esculentum</i> (tomato)	Q7Y0S1, Q05539	II	Sola I chitinase ^g		75, 76
<i>Triticum aestivum</i> (wheat)	Q4Z8L8	II	e		41
	Q8W429	II			
<i>Coffea arabica</i> (coffee) dust	D7REL9	III	Cof a 1		49
<i>Hevea brasiliensis</i> (rubber tree)		III			77, 78
<i>Rubus idaeu</i> (raspberry)		III			48
<i>Zizyphus mauritiana</i> (Indian jujube)	Q2VST0	III	Ziz m 1		46
<i>Cryptomeria japonica</i> (red cedar)	Q5NTA4	IV	e (pollen)		39, 53
<i>Vitis vinifera</i> (grape)	AF532 966	IV	e		11, 39, 53, 79
<i>Zea mays</i> (maize)	ChiA/P29022 ChiB/P29023	IV	e	4MCK	51, 52, 54
<i>Hevea brasiliensis</i> (rubber tree)	Hevein/P02877	chitinase-related	Hev b 6	1HEV, 1Q9B	25, 32
<i>Triticum aestivum</i> (wheat)	agglutinin/isolectin P02876, P10 968	chitinase-related	Tri a 18	7WGA	80

^aChitinases identified as putative allergens are listed according to their classification into different classes. Chitinase-related polypeptides are listed separately. When available, protein names, accession numbers, IUIS allergen names, structure accession numbers in Protein Data Bank are reported.

^bIUIS is the acronym for International Union of Immunological Societies (<http://www.allergen.org/>). ^cEight amino acids are available as the N-terminal sequence. This region shows homology with a class I chitinase (BAD02824) from *Taxodium distichum* (bald cypress). A 26 kDa allergen is recognized by a rabbit antichitinase antibody. Assignment to class I chitinase needs to be confirmed. ^dData in the literature and databases are confusing. The Allergome site <http://www.allergome.org/> reports the existence of a class I chitinase allergen, but contradictory cross-references are reported. The cited Uniprot sequence (P81241) leads to a class II sequence, and the literature citation⁸¹ refers to a class IV chitinase. ^eExperimentally identified as an allergen but not yet added to the IUIS database. ^fFourteen amino acids are available as the N-terminal sequence. This region shows 100% identity with Q6UZ78, a class I chitinase from kidney bean. ^gAllergen is present in the Allergome database and not in IUIS. ^hThe gene is induced by the fungus *Fusarium graminearum*.²⁸

1.³⁶ In this case, a 32 kDa protein from avocado fruit, identified by immune-reaction in the sera of 15 out of 20 avocado allergic patients, was purified and partially sequenced. The corresponding cDNA was also obtained. Analysis of polypeptides and cDNA sequences identified an open reading frame coding for a 326 amino acid protein with a leader peptide of 25 amino acids, a CtBM of 43 amino acids, and additional consensus motifs characteristic of a class I chitinase. The avocado mature chitinase, obtained as a recombinant molecule in *Pichia pastoris* cells, showed the expected endochitinase activity and was effective in inhibiting the binding of allergic patients' IgE to the 32 kDa protein in avocado extracts. Almost simultaneously, other plant chitinases were identified as allergens by procedures including purification of chitinases (with the aid of antihevein or specific antichitinase antibodies for their identification), N-terminal sequencing, immunoblot assays of plant protein extracts with sera of patients allergic to rubber latex, and immunoblot inhibition assays using purified chitinases (or

hevein) as inhibitors. Two polypeptides of 32 and 33 kDa were identified as allergenic chitinases in banana (*Musa acuminata*). N-Terminal sequencing of the purified banana polypeptides revealed high identity (around 80%) with hevein.³⁷ A 32 kDa allergenic polypeptide was also identified as a class I chitinase in chestnut. The reported N-terminal sequence (27 amino acids) was 81% similar to hevein.³⁸ In this case also, the activity of the purified chitinase was assayed. Interestingly, in turnip (*Brassica rapa*), among polypeptides whose expression was enhanced in roots after chemical treatments for the activation of plant defense mechanisms, a class I allergenic chitinase was identified. The same polypeptide was not identifiable with antisera when proteins were extracted from untreated plants.²⁷ A strongly induced allergenic chitinase of class I was also identified in green bean subjected to ethylene treatment.²⁶

Subsequently, a real class I chitinase from *Hevea brasiliensis* was described showing 58% identity between its CtBM and hevein and different IgE binding capacities.³⁹ Recently, a

putative class I allergenic chitinase has been identified in tomato fruits infected with *Pepino mosaic virus* by 2D comparative immunoblot assay with sera of tomato allergic subjects and mass spectrometry analysis.⁴⁰ Skin prick tests conducted with tomato allergic subjects did not, however, show any significant difference between infected and noninfected tomato fruits, indicating that the overall fruit allergenicity was, at least in this case, not appreciably changed by virus infection. More detailed studies have to be performed to clearly establish the allergenic risks due to inducible proteins like chitinases.

Other class I chitinases from plants which have been shown to react with IgE of allergic patients have been reported in kiwi, papaya, obeche, and wheat (Table 1).

Generally, relationships between chitinase three-dimensional structures and allergenicity have not so far received sufficient attention, perhaps due to the assumption that their allergenicity is mainly due to the CtBM. Evidence is emerging, however, indicating that other regions of chitinase molecules can act as allergen epitopes as well (see the Class II section) making a deeper structure–allergenicity analysis of their CM modules important. Knowledge about the structure of class I chitinases comes from a unique work carried out for the CM of the *Brassica juncea* chitinase (EBI accession number Q9SQF7). Even if there are no indications about its allergenicity, its structural analysis can be relevant for possible comparisons with known allergenic chitinases of other classes. The *B. juncea* chitinase is defined as a highly α -helical protein, with a single and irregular β -sheet.²³ The protein has a bilobate shape with a wide cleft in which the active site (Glu-212 and Glu-234) interacts with chitin. The overall structure is stabilized by three disulfide bridges and shows five flexible surface loops (I–V in Figure 1), some of which were located at the border of the active site cleft.

Class II. Chitinases of class II are highly similar to class I enzymes except for the absence of the CtBM module. They have not usually been considered as allergens, according to the assumption that allergenicity of chitinases mainly resides in the hevein-like CtBM. At least this is what emerged from a study on class II chitinases isolated from chestnuts and avocado fruits and assayed against the sera of allergic patients, for whom no IgE binding capacity was identified.³⁸ Data, however, are now emerging which indicate that the CM modules of class II chitinases can be the source of allergenicity. Recently, one class II chitinase has been identified as a possible allergen in wheat flour.⁴¹ By immunoblotting analysis of wheat flour proteins with sera of 22 patients with a suspect history of wheat allergy and high levels of specific IgE, and successive mass-spectrometry analysis of reactive proteins, two chitinases were identified: a 26 kDa protein, which shows high similarity with a class I chitinase from *Hordeum vulgare* (EBI accession number P11955), and an additional chitinase corresponding to a wheat class II chitinase (EBI accession number Q4Z8L8). The purified class II chitinase was reactive to at least 20 out of the 22 available patients' sera. No further details are available about the allergenicity of this molecule. Another wheat class II chitinase (EBI accession number Q8W429) is present in the Allergome database with the code 1274, for which additional information is not available.

Additionally, the Allergome database reports as allergen (code 1273) a class I chitinase from tomato fruits. It corresponds to two almost identical sequences (EBI accession numbers Q05539 and Q7Y0S1) which, however, do not contain the CtBM. The mature polypeptide has an acidic

isoelectric point of 5.6, which further enables its classification among class II chitinases.⁴² For these reasons, the allergen, named Sola 1 chitinase, has been listed in this review among class II chitinases (Table 1).

The structure of class II chitinases has been described for enzymes purified from barley,⁴³ papaya,⁴⁴ and rye,⁴⁵ for which there is currently no information about allergenicity. Also in these cases, as for class I enzymes, the structure is rich in α -helices and shows a substrate-binding groove located between two lobes, with three disulfide bridges contributing to protein folding.

Class III. Class III chitinases are structurally different from class I and class II enzymes and initially not considered as allergens. Recently, however, four plant chitinases belonging to this class have been identified as possible allergens.

The first plant fruit class III chitinase showing IgE binding capacity and IgE-cross reactivity with latex allergens has been identified in Indian jujube (*Zizyphus mauritiana*).⁴⁶ It is a protein of 330 amino acids, including a leader peptide of 25 residues. Both the purified and the recombinant proteins were immunoreactive with sera from latex and Indian jujube allergic patients (seven out of eight patients). This chitinase showed 45.2% identity with hevamine, a class III chitinase present in rubber tree latex, where it had been previously described as an unimportant allergen since only a reduced percentage of examined sera (about 3%) contained reactive IgE.⁴⁷

Mass-spectrometry analysis of IgE-reactive proteins allowed the identification of a class III chitinase also among the allergens extracted from fresh raspberries (*Rubus idaeus*).⁴⁸ As in the case of Indian jujube chitinase and different from hevamine, for raspberry fruits the protein was found to react with most (>80%) of the sera of patients allergic to raspberries. The involvement of plant chitinases of class III in allergic reactions has been recently confirmed by a study conducted on coffee (*Coffea arabica*) allergens.⁴⁹ By screening a phage display library of coffee cDNA with sera of two patients allergic to green coffee beans, a cDNA sequence corresponding to a class III chitinase was identified among those coding for IgE-reactive polypeptides. The recombinant polypeptide (rCofa 1) was subsequently produced and showed immunoreactivity with the sera of the two patients allergic to green coffee and 3 out of 17 sera from workers in coffee industries, all with allergenic symptoms to coffee dust. Immunoreactivity of rCof a 1 with IgE of sera from latex allergic patients was also found.

Currently, there are no studies about three-dimensional structures of class III chitinases among plant food proteins. Only for the nonfood plant chitinase hevamine has the structure been established.⁵⁰ It consists of an $(\alpha/\beta)_8$ barrel fold, different from that described for class I and II chitinases, leading to the assumption of a different structural base for allergenicity.

Class IV. As for class IV chitinases, IgE-binding capacity has been reported in maize kernels, grapes (*Vitis vinifera* and *V. lambrusca*), and wines by immunoblot analysis of plant protein extracts with sera of allergic patients, and subsequent mass spectrometry identification.^{11,51,52} None of these allergens has been currently isolated, preventing further studies of their biological and structural characteristics. Only one chitinase of this class, identified as an allergen in Japanese cedar (*Cryptomeria japonica*) pollen, has been isolated and characterized for its enzymatic activity and allergenicity.⁵³ IgE binding was confirmed in all of the sera of the 31 tested allergic patients, with levels comparable with those observed for the Cry j 1 C.

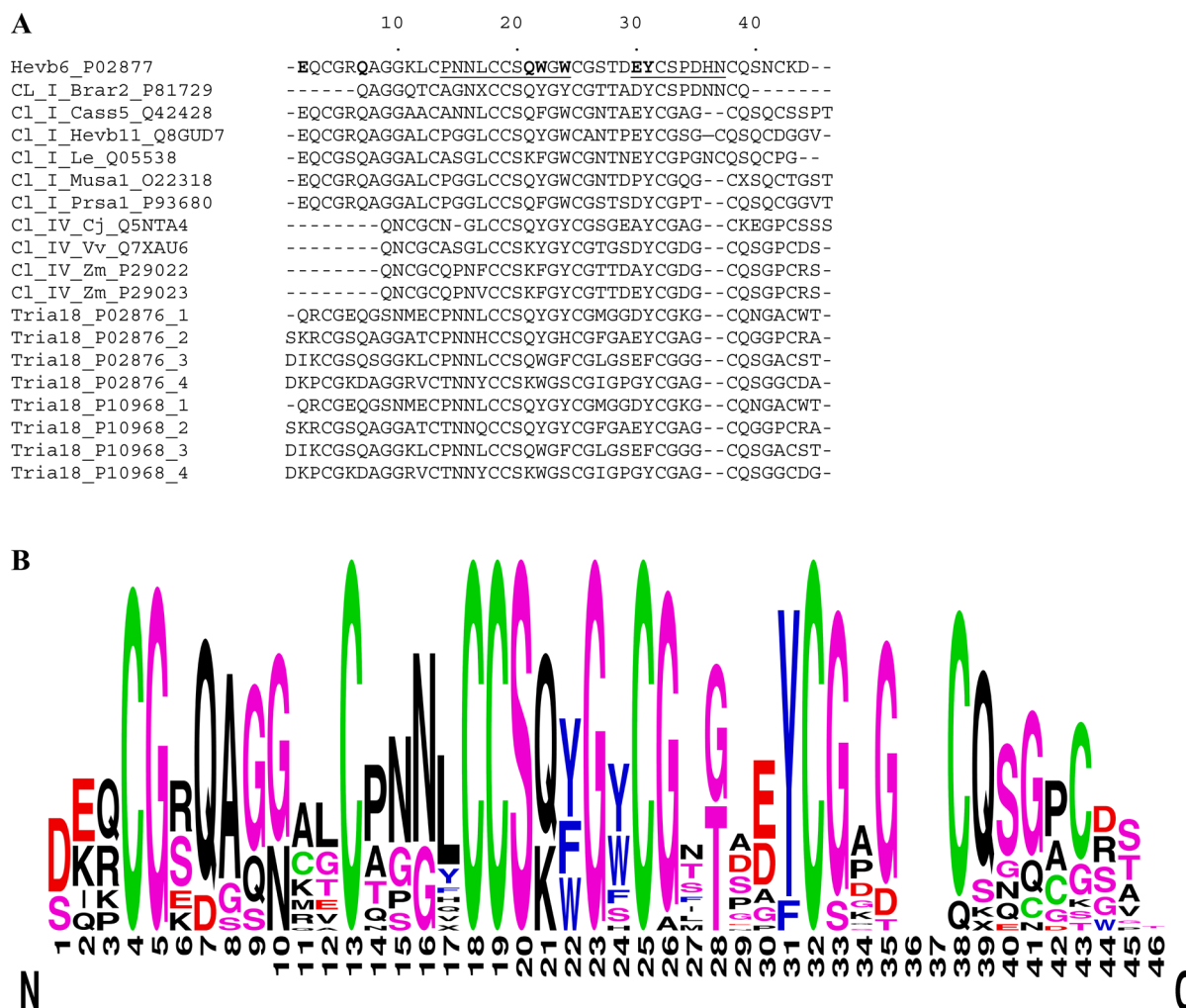


Figure 2. (A) Multialignment of hevein with CtBMs of class I and class IV chitinases and hevein-like regions of WGAs. With the exception of hevein, all the sequences are in the same order as that in Table 1. Class I (CI_I) enzymes are reported before class IV (CI_IV) enzymes. The last eight sequences refer to the chitinase-related polypeptides in Table 1. When possible, the sequence names report the name of the allergen; otherwise, the first letters of the plant's scientific name are indicated. All of the names of sequences contain the EBI accession number. Amino acids of the hevein linear epitopes have been underlined. Trp-21 and Trp-23, together with the other amino acids of the carbohydrate-binding site, are in bold. (B) Graphical representation of the hevein-CtBMs multiple sequence alignment obtained by the WebLogo program⁶⁶ with the following color code: green (C), purple (G, S, T), blue (Y, F, W), and red (D, E).

japonica major allergen. Interestingly, preincubation of the purified molecule with rubber tree latex completely inhibited IgE reaction in pooled sera of *C. japonica* allergic patients.

There are only two structures established for this class of chitinases. The first is that of the Norway spruce (*Picea abies*) chitinase.²⁴ The structure of the CM region shows a high content of α -helices and a small irregular β -sheet, assembled in a bilobate arrangement containing three disulfide bridges and forming a wide cleft exposed on the protein surface. The structure of the CtBM was obtained by homology modeling. It resembles those of class I enzymes and of hevein as well (see also the Other Chitinase-Related Allergens section). More recently, the 3D structure of the putative allergenic chitinase A from maize has also been determined.⁵⁴ In this case, a recombinant truncated form lacking the CtBM was investigated, which shows the same structural components of chitinases of the GH19 family and still retains activity levels comparable with those of the full-length enzyme.

Which module of class IV chitinases (CtBM or CM) is responsible for allergenicity has not been investigated yet. Even

if the presence of a CtBM region, highly homologous to those of class I chitinases and hevein, can be considered as an obvious explanation of their allergenicity, the relatively close structural relationships between their CM modules with that of class II enzymes (among which allergens have been detected) open the possibility that other domains of these proteins could induce allergy. Obviously, this could be the case also for class I chitinases.

Class V. Allergens within this class have not yet been identified. Class V chitinases have been described only in *Arabidopsis thaliana*, *Nicotiana tabacum*, and *Cycas revoluta*, with structures established in the first two cases.^{55,56} Putative chitinases have also been identified in *Medicago truncatula*, rice, and oil palm.^{57,58} Class V chitinases share a similar $(\alpha/\beta)_8$ barrel fold with class III chitinases with which they also share the consensus DXDXE motif containing the catalytic glutamic acid. Other elements, however, clearly distinguish the two classes of chitinases. In particular, class V chitinases have an extra α/β region of about 65–70 amino acids and do not possess six cysteine residues involved in the formation of

disulfide bridges in class III chitinases. In addition, while the sequence identity of molecules within each class is relatively high (around 40% within class III and in the 40–60% range for class V), identity between members of classes III and V is less than 20%. If further confirmed, the absence of allergens within members of class V could provide valuable information to study structural determinants of allergenicity for class III chitinases.

Other Chitinase-Related Allergens. Hevein is the paradigm of chitinase-related allergens. It is a protein of 43 amino acids detected in the latex produced by specialized cells of the rubber tree (*Hevea brasiliensis*), with a possible role as a defense-related protein due to its capacity to bind chitin and inhibit the growth of chitin-containing fungi.⁵⁹ Additionally, thanks to its capacity to bind to glycosylated receptors, a role as a coagulation factor of rubber particles has also been assigned.⁶⁰ Hevein is known as a major allergen present in natural rubber latex which is the cause of allergies among people using latex-derived products, reaching percentages in the range of 3–17% for health-care workers.^{32,61} The hevein gene encodes a polypeptide of 204 amino acids, constituted by a signal sequence of 17 amino acids, the hevein domain, and a 144 amino acid-long carboxy-terminal region showing high homology to wound-inducible potato polypeptides.²⁵ Hevein is rich in cysteine and glycine amino acids and shows high sequence similarities to the CtBM of class I chitinases (higher than 65%), the C-domain of wheat germ agglutinin (WGA) (around 60%), and the CtBM of class IV chitinases (in the 40–60% range). Similar domains have also been detected in other plant proteins (lectins, wound-induced proteins) and fungi (chitinases, toxins, and cell-wall enzymes).

The three-dimensional structure of hevein shows an ovoid shape containing an antiparallel β -sheet and a short four-amino-acid α -helix, assisted by four disulfide bonds.^{62,63} More recent studies, carried out using chemically modified hevein molecules, highlighted the essential role of the surface exposed Trp-21 and Trp-23 amino acids for IgE recognition.⁶⁴ These amino acids are part of the carbohydrate binding site of the molecule and belong to a conformational epitope derived from the structural association of two previously identified linear epitopes.⁶⁵ Most of the aromatic and acid amino acids forming the hevein carbohydrate-binding site are present in other hevein-like molecules identified as allergens, such as WGA and class I and IV chitinases. All of these molecules share the consensus motif C(12)XXXXCCSX φ X φ CGX Ω XAcYC(31) (where X = any amino acid; φ = an aromatic amino acid; Ω = Thr, Ser, or Gly; Ac = Glu or Asp).⁶⁴ Figure 2 reports the alignment of the hevein sequence with the corresponding region of class I and class IV chitinases, and with WGAs.

A recent study based on the cross-reactivity of recombinant hevein and specific plant class I chitinases from banana and avocado fruits, carried out by ELISA assays with sera of latex-allergic patients (for which the involvement in latex-associated plant food syndrome was not known), showed that only subfractions of patients' sera having hevein-specific IgE cross-reacted with banana and avocado chitinases. Also, cases of latex-allergic patients with IgE specific for the chitinases from the two fruits but without specificities for hevein could be observed. Structural analysis of hevein, CtBM-like domains of WGAs, and model structures of class I chitinases clearly indicated that surface variability of allergens is much higher than expected from the alignments of their amino acids sequences,³⁵ making the prediction of cross-reactivity even more challenging.

Conclusions and Perspectives. From the above-reported data, it appears that the general statement that plant chitinases can be allergenic molecules deserves more detailed clarifications. We can assume that plant food chitinases constitute a heterologous group of allergenic molecules, in which at least three different subgroups can be identified: the hevein-like (or CtBM-like) molecules (including both class I and class IV chitinases), the class II chitinases, and the class III chitinases. While a considerable amount of work has been carried out for the identification of class I chitinases as food allergens, chitinases of classes II, III and, IV have been little investigated so far for their potential allergenicity. It is notable that they have been identified as possible allergens in cultivated plants worldwide, widely used in human nutrition, in some cases even as raw food (e.g., salads and fresh fruits).

From a structural point of view, the identification of allergenic molecules belonging to class II chitinases, which do not contain the CtBM module, opens the possibility that the allergenicity of chitinases may not be exclusively due to the CtBM module. This aspect should be properly investigated among class I and class IV chitinases.

Plant chitinases are, therefore, a clear example of molecules for which further studies are required in the near future to better define their allergenic determinants and evaluate their relevance. Recombinant technologies, including techniques based on combinatorial approaches,⁴⁹ combined with advanced structural and physicochemical analysis⁶ can provide useful tools to allergology investigations to firmly establish the structural determinants of allergenicity in chitinases.

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Notes

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ABBREVIATIONS USED

nsLTP, nonspecific lipid transfer proteins; PR, pathogenesis related; GH, glycoside hydrolases; PM, presequence module; CM, catalytic modules; CtBM, chitin-binding module; LM, linker module; WGA, wheat germ agglutinin

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