Review:

Allergy to Mustard Seeds: The Importance of 2S Albumins as Food Allergens

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SUMMARY

Allergy to mustard seeds has been reported for years, in many cases as very strong anaphylactic reactions. Recent studies have shown that this allergy is increasing and that mustard allergens can be considered among the most important food allergens, especially in countries where the consumption of mustard seed is high. Clinical studies describing hypersensitivity to mustard are reviewed in this paper, as well as the detailed structural and immunological characterization carried out on the major allergens from yellow (Sinapis alba) *and oriental* (Brassica juncea) *mustard seeds. These allergens, named Sin a 1 and Bra j 1, respectively, belong to the 2S albumin class of seed storage proteins. Homologous 2S albumins from rapeseed* (Brassica napus) *are also potentially allergenic to mustard-sensitive patients, since they share allergenic determinants with Sin a 1 and Bra j 1. Moreover, in recent years, 2S albumins from several other species have been described as allergens, suggesting that this family of storage proteins is intrinsically allergenic. The availability of three-dimensional data of BnIb, a 2S albumin from rapeseed, has revealed that these proteins are also structurally related to LTPs (lipid transfer proteins) and alpha-amylase/trypsin inhibitors from cereals, which are proteins involved in fruit allergies and baker's asthma disease, respectively. Although their primary structures have significant differences, all these proteins share a common fold of four alpha-helices that are held together by four disulphide bridges. This three-dimensional architecture would therefore have demonstrated itself to be conserved throughout the evolution of such different proteins, and would have given them compactness and stability. These structural features are discussed in terms of their possible relationship with the allergenic character of these groups of plant proteins.*

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KEYWORDS

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INTRODUCTION

Type I allergies are common clinical disorders in developed countries, food being one of the most relevant biological sources of these atopic diseases. Many seed spices have been shown to be allergenic, and mustard is one of the most significant in terms of its widespread use and allergenic potency. The prevalence of food allergy varies with the dietary practice of different populations and in countries such as France, where mustard is extensively consumed, hypersensitivity to mustard seeds is increasing and has been included among the most important food allergies (Andre et al. 1994, Rance et al. 1999, Rance et al. 2000). In addition, several authors have reported on its allergenic potency and this is of special relevance if we consider that mustard is frequently present as an additive to many meals or as hidden allergens in commercial foods (Panconesi et al. 1980, Niinimaki & Hannuksela 1981, Monreal et al. 1992, Kanny et al. 1995, Rance et al. 2000). Table 1 summarizes foods in which mustard can be found. Although no reports on fatal anaphylactic reactions to mustard seeds have been described, its allergenic potency must be seriously taken into account. In fact, allergens from other seeds and nuts, such as peanuts, walnuts or Brazil nuts, have been described as responsible for causing fatal or near-fatal reactions in hypersensitive patients (Sampson et al. 1992). Interestingly, these and other nuts contain allergens that have been related to those of mustard seeds (Teuber et al. 1998).

Table 1: List of foods in which mustard can be found

Babies and toddlers commercial foods
Barbecue sauce
Curry sauce
Cumberland sauce
Flours for flavouring fried fish or meat
Ketchup / Tomato sauce
Lubricant
Marinades
Mayonnaise
Meat (processed)
Meat (sausages)
Mustard powder (additive to foods)
Mustard sauce (commercial)
Mustard paste (home-made, combining the powder with wine or vinegar and oil)
Piccalilli / Mustard pickle
Salad dressing
Salad oil
Spices for flavouring
Vinagrettes

The information included in this table is taken from the clinical cases reviewed in this paper (Table 2) and from the Food Additive & Preservatives Allergy & Intolerance Database (FAP AID) designed by Dr. Harris Steinman (http://zingsolutions.com/food/index.html#info)

The major allergens of mustard seeds are storage proteins of the 2S albumin class, which are abundant proteins of these seeds. They are small (12 to 15 kDa) and basic proteins, generally composed of two different polypeptide chains (of about 3-5 and 8-10 kDa) linked by disulphide bridges. Allergenic 2S albumins have been isolated and characterized from two species of mustard: yellow or white mustard (*Sinapis alba* L.) (Menéndez-Arias et al. 1987, Menéndez-Arias et al. 1988) and oriental mustard (*Brassica juncea* L.) (González de la Peña et al. 1991). These two allergens have been named Sin a 1 and Bra j 1, respectively, according to the nomenclature recommended by the World Health Organization and the International Union of Immunological Societies (King et al. 1994). Apart from these two species, black mustard (*Brassica nigra* L.) is also frequently used as condiment (Meding 1985, Monreal et al. 1992). Table mustard is made from varying mixtures of crushed seeds from different species according to the country of origin or the manufacturer. For example, yellow and black mustard are the commonest used in Europe, while the oriental mustard flour is most abundant in mustard extracts from the USA or Japan (González de la Peña et al. 1991).

S. alba, *B. juncea* and *B. nigra* belong to the *Brassicaceae* family, which also includes others such as *Brassica napus* (rapeseed), *Raphanus sativus* (radish) and *Arabidopsis thaliana* (thale cress). The sequences of many 2S albumins from these species have been determined and studies on their gene expression regulation and post-translational processing have been carried out (Crouch et al. 1983, Ericson et al. 1986, Krebbers et al. 1988, Monsalve & Rodríguez 1990, Raynal et al. 1991). Many reports refer to

these storage proteins as "napins" or napin-like proteins, since *B. napus* is the species most extensively studied. One of its members, napin BnIb, has been isolated and structurally characterized by our group, obtaining the first data on the three-dimensional organization of a 2S albumin (Monsalve et al. 1991, Rico et al. 1996). Besides the interest in these proteins for their storage function and their allergenicity in mustard seeds, 2S albumins have also been studied because of their antifungal activity (Terras et al. 1993, Byczynska & Barciszewski 1999) and also because of their nutritional qualities, since some of them have an unusually high content of essential amino acids. As an example, the 2S albumins of the Brazil nut (*Bertholletia excelsa*, family *Lecythidaceae*) are enriched in methionine (Gander et al. 1991) and have been used to improve the nutritional quality of other seeds (Guerche et al. 1990, Saalbach et al. 1994, Nordlee et al. 1996). Nevertheless, in the case of transgenic soybean, the seeds obtained exhibited allergenic properties due to the presence of 2S albumins expressed by transferred DNA from Brazil nuts (Nordlee et al. 1996). As a matter of fact, 2S albumins from different species have been described as allergens, as will be shown later in this review.

Many reports on mustard hypersensitivity can be found in the literature, but there has not been a review on mustard allergens. In this manuscript we intend to survey the most relevant clinical studies on this food allergy, as well as reviewing the biochemical and immunological studies accumulated to date on the major mustard allergens. The structural relationship of the major mustard allergens to other allergenic proteins is presented and discussed in terms of possible common features that could be related to their allergenic character.

CLINICAL DATA ON MUSTARD SEED ALLERGY

Mustard allergy has been reported for years. Most of these studies have analysed clinical cases of a very limited number of patients with hypersensitivity symptoms after having ingested foods containing table mustard or after inhaling or coming into contact with this spice as a powder. Table 2 summarizes these studies and shows that most of them use skin prick testing (SPT) and/or radioallergosorbent test (RAST) to confirm the allergenicity to mustard seeds. Few studies have used histamine release assays and only recently have oral challenges been carried out.

In spite of the relatively low number of individuals affected, many authors have emphasized the severity of symptoms caused by mustard seed allergens (Stricker et al. 1986, Monreal et al. 1992, Andre et al. 1994, Jorro et al. 1995), which induce strong anaphylactic reactions that need urgent clinical intervention. This fact is of importance considering that mustard is present in many foods (Table 1) and that very often mustard products are hidden in foods (Panconesi et al. 1980, Andre et al. 1994, Kanny et al. 1995, Rance et al. 2000). The severity of symptoms caused by mustard has led some authors to suggest not using oral challenges for testing this type of food allergy (Stricker et al. 1986, Jorro et al. 1995). They state that SPT and RAST are sufficient to demonstrate allergenicity, and systemic reactions can be avoided in this way.

The most complete clinical studies on mustard allergy have been those of Rance and colleagues (Rance & Dutau, 1997, Rance et al. 1999, Rance et al. 2000). They tested many food allergens in a selected population of 722 French children and adolescents (aged from one month to 15 years) that were allergic to foods. Their work represents the first analysis in which oral challenge is used to demonstrate mustard allergy. Rance and Dutau (1997) use a labial food challenge (LFC) test as a reliable technique to confirm the allergenicity of food in individuals suspected to be hypersensitive from their case history, positive SPT and specific serum IgE assays. It is a simple and rapid method to perform, and it is associated with only low risks of systemic reactions. Positive results clearly indicate the existence of food allergy, but the sensitivity of this method is its major drawback: about 23% of mustard allergic individuals had to be confirmed by single-blind placebo-controlled food challenges (SBPCFC, Rance & Dutau, 1997). In Rance et al. 2000, the authors state that the strong taste of mustard makes it very difficult to perform double-blind placebo-controlled food challenges (DBPCFC), and that the use of lyophilized capsules to circumvent this problem would prevent the first oropharyngeal contact. Rance and colleagues mention that

Table 2: Chronological compilation of case reports and clinical studies on mustard allergy
Data on clinical symptoms, number of allergic patients (with respect to the population tested, when applicable) and clinical tests carried out are included. Abbreviations used: SPT = skin prick testing; RAST = Radioallergosorbent test (generally Phadebas Pharmacia); LFC = Labial Food Challenge; SBPCFC = single-blind placebo-controlled food challenge; REIA = reversed enzyme immunoassay.

Reference	Clinical Symptoms of patients	No. patients	SPT	RAST	Other comments
Panconesi et al. 1980	Acute giant urticaria with oedema of the glottis	1	Yes	Yes	Mustard present as contamination
Meding 1985	Vesicular hand eczema	1	Yes	--	
Widstrom & Johansson 1986	Recurrent cases of acute severe generalized urticaria and angioneurotic oedema	1	Yes	Yes	
Stricker et al. 1986	Severe anaphylactic symptoms	3	Yes	--	
Dannaker & White 1987	Contact dermatitis symptoms (vesicular dermatitis)	1	Yes	--	Allergy ascribed to isothiocyanates. Patch tests were used.
Kavli & Moseng 1987	Contact urticaria	3	Yes	--	Also patch tests were performed. Patients with occupational dermatitis
Menéndez-Arias et al. 1988 and 1990	Bronchial asthma and allergic rhinitis. Anaphylactic shock in two patients.	18 out of 107	Yes	Yes	Also REIA and histamine release assays
Niinimaki et al. 1989	Gastric pain, rhinitis, atopic dermatitis, or birch pollen allergy (in the whole population)	29 out of 50	Yes	Yes (only in 4 cases)	Population with hypersensitivity to spices. Cross-reactivity to birch pollen allergy proposed.
Diamond et al. 1990	Contact dermatitis	1	--	--	Patch testing with different parts of the plant (not with the seeds). Reaction adscribed to oil of mustard
Domínguez et al. 1990	Angioedema, urticaria or anaphylactic shock	7	Yes	Yes	Also REIA and histamine release assays
Kohl & Frosch 1990	Irritant contact dermatitis	1	Yes	--	Temporary sensitization. Negative SPT.
Vidal et al. 1991	Acute generalized urticaria, facial and throat swelling, chest tightness	2	--	Yes	
Monreal et al. 1992	Anaphylactic reaction symptoms	2	Yes	Yes	
Malet et al. 1993	Anaphylactic reaction symptoms	2	Yes	Yes	Also histamine release and nasal provocation tests
Andre et al. 1994	Anaphylactic reaction symptoms	2 out of 60	Yes	Yes	
Niinimaki et al. 1995	Atopic dermatitis with respiratory symptoms; respiratory symptoms; atopic dermatitis only; chronic hand eczema	22 out of 49	Yes	Yes	Patient population with strong SPT to spices
Kanny et al. 1995	Oral pruritus, oedema and generalized urticaria	1	Yes	Yes	
Jorro et al. 1995	Anaphylactic reaction symptoms	3	Yes	Yes	

Monsalve et al. 1997	Wheezing with shortness of breath. Oedema and pruritus in the lips, oral mucosa and the pharynx, and facial urticaria	1	Yes	--	Also direct and inhibition ELISA and immunoblotting
Rance & Dutau 1997 Rance et al. 1999	Atopic dermatitis. Laryngeal oedema, angioedema, asthma and anaphylaxis. Oral allergy syndrome	23 out of 142 49 out of 544	Yes	Yes	LFC and SBPCFC. Population of children and adolescents
Rance et al. 2000	Atopic dermatitis. Urticaria and/or angioedema. Asthma. Laryngeal oedema with oral alllergy syndrome.	15 out of 36	Yes	Yes	SBPCFC to diagnose allergy (the 36 children were positive by SPT and/or RAST)

mustard allergy is increasing in France, and that it affects to more than 10% of food-allergic patients. They also state that this allergy is among the five most important food allergies. In fact, eggs, peanuts, mustard, cow's milk and cod, in this order, are responsible for 78% of the cases of food hypersensitivity in their studies. These studies have also shown that allergic reactions to mustard start early in life, usually below three years of age. Sensitization could take place *in utero* or through breast-feeding, and frequent contact with mustard in infancy could be explained by the presence of mustard in baby foods in glass pots for babies and commercial foods for toddlers (Rance et al. 2000). Therefore they suggest that mustard should be systematically included in screening tests for food allergies and that labelling of foods must be improved, since mustard is often hidden in food.

CHARACTERIZATION OF MUSTARD SEED ALLERGENS AND RAPESEED HOMOLOGOUS PROTEINS

Sin a 1, the major allergen of yellow mustard (*Sinapis alba*) seeds was identified and characterized as a storage protein of the 2S albumin class by our group (Menéndez-Arias et al. 1987, Menéndez-Arias et al. 1988). The proteins that can be found in mustard seeds are mainly 12S globulins and 2S albumins. They were fractionated by size exclusion chromatography, and tested for their allergenicity using sera of mustard-sensitive patients by reverse enzyme immunoassays (REIA), histamine release and RAST inhibition assays. The 2S albumin fraction was found to be the most potent allergen in the extract, while the 12S fraction had a much lower response (Menéndez-Arias et al. 1988). Sin a 1 was isolated from the 2S albumin fraction by ion exchange chromatography and was characterized structurally and immunologically. A protein with very similar structural and immunological characteristics was obtained from oriental mustard (*Brassica juncea*) seeds and named Bra j 1 (González de la Peña et al. 1991).

Sin a 1 was immunologically mapped by competition and complementation assays using ten specific monoclonal antibodies (Menéndez-Arias et al. 1990). According to these studies, Sin a 1 contains two immunodominant regions to which most of these monoclonal antibodies are directed. These regions are also allergenically relevant, since these IgG antibodies inhibited IgE binding to Sin a 1 to some extent. Most of the monoclonal antibodies bound to conformational antigenic determinants, and this was also the case for the IgE binding epitopes. Nevertheless, one of the monoclonal antibodies (named 2B3 in Menéndez-Arias et al. 1990) recognized a continuous epitope on the large chain of Sin a 1. More defined studies led us to the conclusion that this specific region contained an important allergenic determinant of the molecule (Monsalve et al. 1993). It was also observed that the epitope recognized by 2B3 was specific to mustard allergens (Sin a 1 and Bra j 1), since this monoclonal antibody did not recognize the most abundant 2S albumin of rapeseed (Bra n 1, as it has recently been named in accordance with the IUIS Allergen Nomenclature Subcommittee, King et al. 1994; this protein corresponds to napin BnIII in Monsalve & Rodríguez, 1990). The difference in recognition of 2B3 was explained by the variation in the primary structures of Sin a 1, Bra j 1 and Bra n 1 (Monsalve et al. 1993), particularly by a histidine residue which is present only in the mustard allergens (it corresponds to position 118 of the aligned sequences of Figure 1, in which these three proteins correspond to positions (a), (b) and (c), respectively). The region to

which this antibody binds is located in a segment that has been named the "hypervariable region" of the 2S albumins (Krebbers et al. 1988, Monsalve et al. 1991, Raynal et al. 1991), because of the high variability between the different members of this family in this stretch of the sequence. Figure 1 clearly shows this variability: very long and variable gaps have to be opened by the computer program, between positions 100 to 120 of the aligned sequences, to achieve the best alignment (the other big gap which appears in Figure 1 corresponds approximately to the limits of the small and large chains that constitute most of these 2S albumins). Our group also emphasized the antigenic relevance of this region after obtaining a three-dimensional model of Sin a 1, using the atomic coordinates of napin BnIb as reference (Monsalve et al. 1995, Rico et al. 1996). In both molecules, this segment corresponds to an exposed loop which links two of the alpha-helices that make up the scaffold of these molecules (Figure 2). The sequence variability of this loop, which is evolutionarily allowed, would mean that the folding process and the core structure are independent of its length, as suggested by Krebbers et al. (1988), and it constitutes an important antigenic region of the 2S albumins.

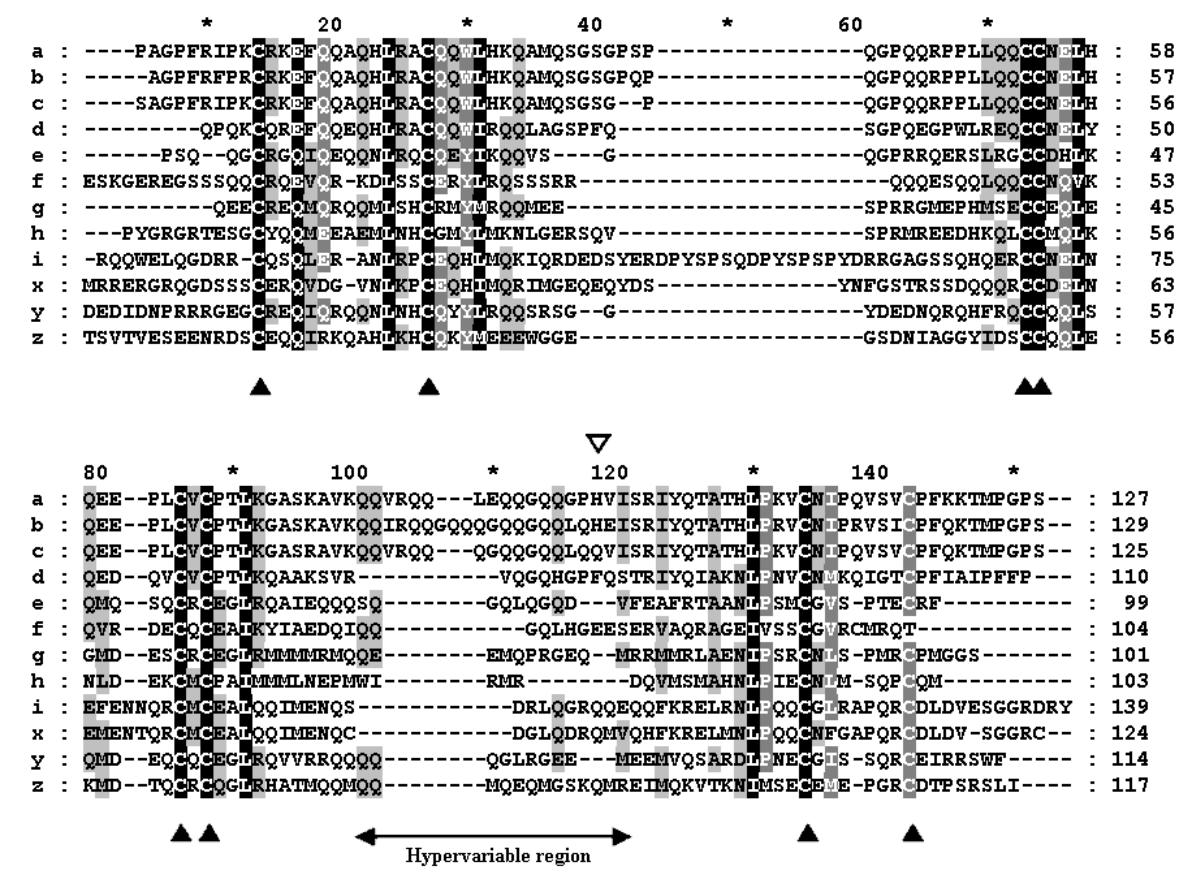

Figure 1: Multiple sequence alignment of allergenic 2S albumins

Letters on the left stand for the following allergens (in correspondence with data in Table 3): (a)-Sin a 1; (b)-Bra j 1; (c)-Bra n 1; (d)-BnIb; (e)-Ric c 1; (f)-Ric c 3; (g)-Ber e 1; (h)-SFA8; (i)-Ara h 2; (x)-Ara h 6; (y)-Jug r 1; (z)-Mat5-D. Sequences of the mature proteins (generally small and large chains together) were used for this alignment, except for sequences (x), (y) and (z), for which only DNA data are known (the deduced amino acid sequences were cut at the N-terminal ends to fit the length of the longest mature protein). Filled triangles show the consensus cysteine pattern of 2S albumins and the open triangle shows a specific position mentioned in the text. Numbers on the right of the alignment correspond to the sequence position of each molecule. ClustalW was the program used (Thompson et al. 1994) and dashes represent gaps opened for the best alignment. Shadowing of columns represents conservation among all the sequences: darker backgrounds stand for the highest values of conservation.

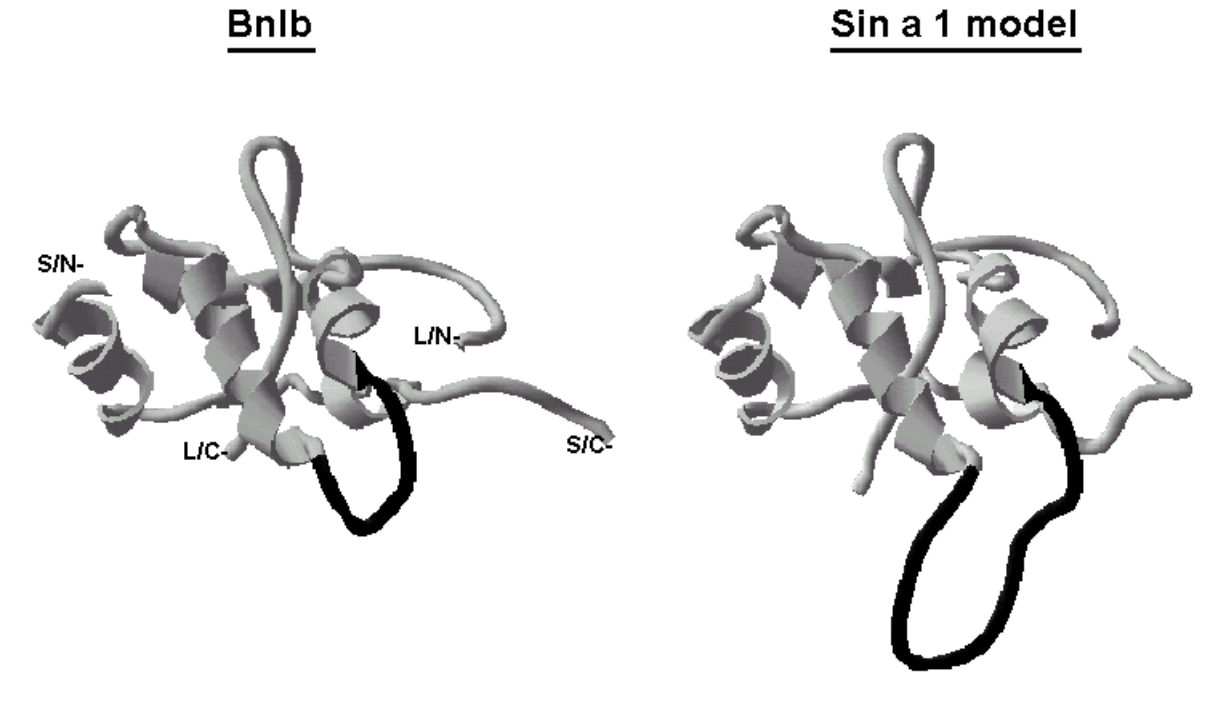

Figure 2: Schematic ribbon representation of napin BnIb (PDB code: 1PNB) and Sin a 1 model (Monsalve et al. 1995) The loop that corresponds to the hypervariable region is drawn in black. Small and large chains' termini of BnIb are labelled as S/N- and L/N- for the NH_2-terminal, respectively, and S/C- and L/C- for the COOH-terminal, respectively.

Other antigenic and allergenic determinants are yet to be studied in detail, and to this end our group has obtained recombinant forms of Sin a 1 (rSin a 1) or closely related counterparts of the 2S albumin class (González de la Peña et al. 1993, González de la Peña et al. 1996, Villalba et al. 2000). The importance of obtaining recombinant products, apart from their being useful tools for the diagnosis and specific immunotherapy of allergy or for studying structural features of proteins, is evident in the case of mustard allergens, since 2S albumins are a very complex protein fraction codified by multigenic families, each one therefore represented by multiple isoforms. Recombinant production avoids manipulation of microheterogeneous natural proteins. rSin a 1 obtained as a fusion protein was shown to have most of the antigenic and allergenic determinants of natural Sin a 1 (González de la Peña et al. 1996), although the yield of soluble protein obtained was low. Recently, the cloning and expression of recombinant BnIb (rBnIb) precursor was achieved in the yeast *Pichia pastoris* (Villalba et al. 2000). In this case, a high yield (50 mg of protein per litre of culture) of a properly folded molecule was obtained. The immunological and structural features of rBnIb were equivalent to those of the natural protein. The recombinant molecule was bound by 50% of the mustard-sensitive sera tested and therefore shares allergenic determinants with mustard allergens. The availability of these recombinant molecules, as well as of modified derivatives, will help to better define the IgE-epitopes of mustard allergens and should also facilitate the design of hypoallergenic variants which could be used in specific immunotherapy.

Our group has also identified and characterized the major allergen of rapeseed (*Brassica napus*), Bra n 1 (Monsalve et al. 1997). This protein is a close homologue of Sin a 1, with 94% of sequence similarity after their alignment (Figure 1, Table 3). Bra n 1 has been defined as an occupational allergen that affected a patient who worked in a feed processing plant, handling rapeseed flour, cereals, soybean, and sunflower seeds, among other foods. The patient gradually became hypersensitive to rapeseed flour, and the disease developed into a strong food hypersensitivity to mustard-containing products. The antigenic properties of Bra n 1 were studied, in comparison to those of Sin a 1, by using sera from mustard- and rapeseed-sensitive patients. These studies allowed us to conclude that these two proteins share common antigenic

and allergenic determinants. Several authors had previously reported on the cross-reactivity between the proteins of rape and mustard seeds (Meding, 1985, Widstrom & Johansson, 1986, Monreal et al. 1992). Therefore, homologous proteins of Sin a 1 and Bra j 1 are also potentially allergenic for mustard-sensitive patients.

Table 3: Sequence and molecular data of 2S albumins that have been defined as allergens

Sequence data of mature proteins are included whenever possible, complemented by data on the precursors deduced from cDNAs when these are complete. Accession numbers correspond to SwissProt and TrEMBL databases, except for L77197, which corresponds to Genbank. The proteins are listed in the same order as that of Figure 1, with the same reference letter.

Allergen Name	Species and common name	Database Accession Number	Precursor Length (*)	Mature Chains' Size (**)	Mr of mature protein (Da)	Ref. (***)	Ident.% (Simil.%)
Sin a 1	*Sinapis alba* (yellow mustard)	P15322		39 / 88	14180	(a)	(****)
Bra j 1	*Brassica juncea* (oriental mustard)	P80207		37 / 92	14644	(b)	86 (91)
Bra n 1	*Brassica napus* (rapeseed)	P80208		37 / 88	14035	(c)	92 (94)
BnIb	*Brassica napus* (rapeseed)	P24565		31 / 79	12691	(d)	47 (61)
Ric c 1	*Ricinus communis* (castor bean)	P01089	258 (*)	34 / 65	11212	(e)	25 (43)
Ric c 3	*Ricinus communis* (castor bean)	P01089	258 (*)	37 / 70	12032	(f)	20 (35)
Ber e 1	*Bertholletia excelsa* (brazil nut)	P04403	146	28 / 73	12218	(g)	18 (39)
SFA8	*Helianthus annuus* (common sunflower)	P23110	141	103	12155	(h)	14 (28)
Ara h 2	*Arachis hypogaea* (peanut)	L77197	157	138	16637	(i)	14 (31)
Ara h 6	*Arachis hypogaea* (peanut)	Q9SQG5	129			(x)	17 (34)
Jug r 1	*Juglans regia* (English walnut)	P93198	139			(y)	21 (38)
Mat5-D	*Gossypium hirsutum* (upland cotton)	Q39787	139	(27 / 76)		(z)	12 (31)

Footnotes:

(*) Size expressed as number of amino acids (only shown when the complete precursor is known). Ric c 1 and Ric c 3 are codified by the same precursor.

(**) Defined as the number of amino acids of the small and large chains. In the case of SFA8 and Ara h 2, only one chain constitutes the mature protein. For Mat5-D, the predicted size of chains is shown.

(***) Letters used to identify the different allergenic proteins correspond to the following bibliographic references: (a) - (Menéndez-Arias et al., 1988); (b) - (Monsalve et al., 1993); (c) - (Monsalve et al., 1997); (d) - (Monsalve et al., 1991, Villalba et al., 2000); (e) and (f) - (Irwin et al., 1990, Bashir et al., 1998); (g) - (Ampe et al., 1986, Nordlee et al., 1996); (h) - (Kelly & Hefle, 2000); (i) - (Stanley et al., 1997); (x) - (Kleber-Janke et al., 1999); (y) - (Teuber et al., 1998); (z) - (Youle & Huang, 1979, Galau et al., 1992).

(****) Identity (Ident.%) and similarity (Simil.%) percentages correspond to the alignment shown in Figure 1 and are referred to the pairwise comparison with Sin a 1. Identities correspond to exact matches and similarities to conservative changes.

STRUCTURAL FEATURES AND ALLERGENICITY OF 2S ALBUMINS

In the last few years, 2S albumins from other species have also been described as allergens, as is shown in Table 3. The presence of these allergenic proteins in seeds of numerous species has led to the proposal that these storage proteins can be considered "universal allergens" (Teuber et al. 1998). This is so even if they do not necessarily cross-react and even though their primary structures differ considerably in some cases. As is shown in Table 3 and Figure 1, the percentage of sequence identity of some of these 2S albumins is as low as 14%. This is the case with SFA8, a sunflower 2S albumin, and Ara h 2, a major allergen of peanut that has a prevalence of 90% in patients allergic to peanut (Stanley et al. 1997). These two proteins are in the borderline of being considered homologous to Sin a 1 (their percentages of similarity reach 29 and 31%, respectively). Both SFA8 and Ara h 2 are the only allergenic 2S albumins that have been described as being constituted of a single polypeptide chain. In any case, heterodimeric 2S albumins are synthesized as single precursors that are proteolytically cleaved during maturation with the loss of an internal peptide, apart from the removal of other N- and C-terminal regions. The alignment shown in Figure 1 has been carried out with the sequences of mature allergenic 2S albumins, except for the sequences (x), (y) and (z), which correspond to the unprocessed precursors of these proteins. Hence, the identity and similarity values shown in Table 3 for these proteins are lower than the ones that could be obtained with the mature proteins.

In spite of the differences mentioned, the evolutionary relationship of 2S albumins has been pointed out by several authors (Raynal et al. 1991, Bashir et al. 1998, Teuber et al. 1998). In fact, one constant feature in all the storage proteins belonging to the 2S albumin class is the presence of cysteine residues distributed according to a conserved pattern (...C...C... / ...CC...CxC...C...C...; see Figure 1). These residues generally form four disulphide bridges that covalently link both polypeptide chains, and are also present in the one-chain SFA8 (Egorov et al. 1996). The existence of this cysteine pattern has also been described in other storage proteins, such as the sulphur-rich prolamins and alpha-amylase inhibitors from cereals (Kreis et al. 1985, Shewry et al. 1995). Therefore, the existence of a common ancestor has been proposed not only for the 2S albumins, but also for other groups of storage proteins belonging to groups phylogenetically more distant.

The determination of the global fold of napin BnIb (Rico et al. 1996), the only 2S albumin for which three-dimensional data are available, revealed that these storage proteins are structurally similar to two different families of proteins: the non specific Lipid Transfer Proteins (LTPs) and the cereal alpha-amylase/trypsin inhibitors (Rico et al. 1996, Behnke et al. 1998). As a matter of fact, these three groups of proteins constitute a super-family of the "all alpha" protein class, according to the classification of the SCOP structural database (Murzin et al. 1995). These three families are defined as disulphide-rich, with a common fold of "four helices, folded leaf, right-handed superhelix". There is no apparent relation among the primary structures of these proteins, but if they are aligned considering their three-dimensional organization, it is possible to match a cysteine pattern that resembles the same distribution mentioned above. The cysteine residues that constitute this pattern have also been described as being involved in disulphide bridges in a similar distribution to that of the 2S albumins (Egorov et al. 1996, Rico et al. 1996, Poznanski et al. 1999). These structural similarities have led different authors (Rico et al. 1996, Behnke et al. 1998, Pandya et al. 2000) to suggest the existence of a common ancestor in plants that must have diverged to proteins with different functional activities but with a conserved common conformation. This proposal is of utmost interest if we consider that, apart from the 2S albumins, many members of each of these families have been defined as allergenic proteins. An important case is that of the pan-allergenic LTPs, which are very potent allergens present in different fruits (Asero et al. 2000). Belonging to the family of the LTPs, the hydrophobic protein of soybean (HPS) from the hull of *Glycine max* seeds has also been defined as an allergen (Baud et al. 1993, González et al. 1995). On the other hand, many studies have reported that the allergens that induce baker's asthma disease are seed proteins belonging to the alpha-amylase/trypsin inhibitors family (Barber et al. 1989, Gómez et al. 1990, Izumi et al. 1992, García-Casado et al. 1995, Behnke et al. 1998).

The structural relationship between such a wide group of important plant allergenic proteins opens the question of whether there are any structural features that can be related to their allergenic character. Their compactness due to the disulphide bridges packing would help to give to these proteins a special resistance to proteolysis and thermal denaturation. This property has been observed for 2S albumins (Domínguez et al. 1990, Oñaderra et al. 1994, Astwood et al. 1996) and also for LTPs (Asero et al. 2000). In the case of food allergens, this could allow these proteins to reach gastrointestinal tract almost intact. In fact, digestibility has been emphasized as a key factor for food allergens (Astwood et al. 1996, Aalberse, 2000). Moreover, their allergenicity could be favoured by the interaction of these proteins with membranes. LTPs clearly interact with membranes due to their functionality (Wirtz, 1991, Poznanski et al. 1999). In the case of 2S albumins, we have demonstrated that there is a strong interaction of Sin a 1 with phospholipid vesicles (Oñaderra et al. 1994). In this work we proposed that this interaction could underlie its allergenic character as a food allergen. In fact, this could result in an increased cellular uptake, a reduced neutralization by secretory antibodies and a decreased degradation in the blood stream.

Finally, it is also worth mentioning that, to our knowledge, no cross-reactivity between these groups of allergenic proteins has been described. Their common global fold, which is kept compact by a similar network of disulphide bridges, has regions of sequential variability located mainly in the loop regions (Rico et al. 1996). This variability could be the reason for the lack of cross-reactivity between them. This is in agreement with Rod Aalberse, who states that proteins with a similar fold are not necessarily cross-reactive (Aalberse, 2000).

The elucidation of these questions will require many more studies. Future structural and immunological studies in this wide group of allergenic proteins will certainly reveal which of these structural properties, if any, are the most significant for inducing allergic reactions and could give some clues to enhance understanding of the molecular basis of food allergy.

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[Summary] [Abbreveations]
