

Cloning, sequencing, and recombinant production of Sin a 2, an allergenic 11S globulin from yellow mustard seeds

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Background: Among allergenic spices, mustard represents 1 of the most important in terms of allergenic potency and widespread use. An 11S globulin (Sin a 2) has been isolated from yellow mustard seeds and identified as a new major allergen.

Objective: Cloning and sequencing a cDNA encoding a Sin a 2 subunit and producing the allergen as a recombinant protein.

Methods: Sin a 2 subunit-encoding cDNA was amplified by polymerase chain reaction, cloned, and sequenced. The allergen was produced as a recombinant protein in *Escherichia coli* and used for enzyme-linked immunosorbent assay, immunoblotting, and inhibition experiments. Sera from patients with mustard allergy and an anti-polyhistidine monoclonal antibody were used.

Results: Sin a 2-specific cDNA comprises an open reading frame that encodes a protein of 510 amino acids, in which the first 23 residues correspond to the signal peptide. Sequence alignment with other allergenic 11S globulins showed levels of sequence identity ranging between 27% and 38%. Three peptides described as epitopes in Ara h 3 were moderately conserved in Sin a 2. Approximately 87% of the IgE binding to natural Sin a 2 was inhibited by the recombinant allergen using sera from patients with mustard allergy.

Conclusion: The recombinant 11S globulin from yellow mustard seeds produced in *E coli* retained the IgE-binding capability of the natural allergen.

Clinical implications: The availability of Sin a 2 sequence and its recombinant production could help to develop future therapeutic approaches and might well open new investigation lines to resolve whether 11S globulins are proteins implicated in cross-reactivity processes involving mustard seeds. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Mustard allergy, Cupin superfamily, 11S globulin, Sin a 2, recombinant allergen, cross-reactivity

Recent studies have revealed that up to 7% of young children and around 4% of adults suffer from some type of food allergy.¹ Among spices, mustard constitutes 1 of the most frequent causes of allergy. Immediate skin reactions, angioedema, and anaphylactic shocks have been reported in hypersensitive patients after the ingestion of mustard seed flour or manufactured foods containing this spice.²⁻⁹ Mustard is extensively consumed in homemade meals and frequently contained as a hidden condiment in sauces, salad dressings, or flavoring powders, which makes avoidance measures difficult and increases the risk of suffering adverse reactions.²⁻⁷ Therefore, mustard has been recently included in the list of 12 potential allergenic foods to be labeled in a European Union directive on labeling of foods.¹⁰

Over the last 2 decades, allergenic 2S albumins from yellow (*Sinapis alba*) and oriental (*Brassica juncea*) mustards—Sin a 1 and Bra j 1, respectively—have been the single allergens reported in mustard seeds.^{11,12} Recently, an 11S globulin (Sin a 2) from yellow mustard seeds has been identified as a new major allergen by proteomic tools (2-dimensional electrophoresis, mass spectrometry [MS], and Edman degradation).¹³ The subunit of the allergen is a basic 51-kd protein composed of 2 polypeptide chains of 36 kd and 23 kd apparent molecular masses, which can be separated by reductive treatments. Both separate chains retained IgE-binding capability.¹³ 11S globulins, also named legumins, are salt-soluble storage proteins from seeds and belong to the cupin superfamily. Several major allergens belonging to this protein family have been characterized from soybean (glycinins G1-G2),¹⁴ peanut (Ara h 3),¹⁵ hazelnut (Cor a 9),¹⁶ Brazil nut (Ber e 2),¹⁷ and cashew nut (Ana o 2).¹⁸ Little evidence still exists of IgE cross-reactivity processes involving this family of proteins with an overall sequence identity under 40%. However, cross-reactivity between coconut and walnut has been attributed to 11S globulins.¹⁹

It has been reported that more than 50% of the patients with mustard allergy present hypersensitivity to other vegetable foods, mainly nuts and legumes.⁹ Whether 2S albumins (Sin a 1) and 11S globulins (Sin a 2) might be allergens implicated in these processes remains unresolved.

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TABLE I. Oligonucleotides used as primers in the PCR cloning strategy

Oligomer	Nucleotide sequence*	Amino acid sequence
SIN2-A	5'-AARGGNCCNTTYCARGT-3' (sense)	KGPFQV
SIN2-B	5'-DATYTGRAANGCRTTNGT-3' (antisense)	TNAFQI
SIN2-C	5'-ACCAACGCTAACGCGATG-3' (sense)	TNANAM
SIN2-D	5'-GGTGTAGCTGTTGACGCTGGT-3' (antisense)	TSVNSYT
SIN2-E	5'-GTCCAGTCGCTAGGGGTT-3' (sense)	VQSLGV
SIN2-F	5'-TCAAACCTCGACTATCTCCTC-3' (antisense)	EEIVEV
UPM	5'-CTAATACGACTCACTATAGGGCAAGCAGTGGTAACAACGCAGAGT-3'	—

*In the nucleotide sequence, R, D N, and Y stands, respectively, for A/G, G/A/T, A/C/G/T, and C/T.

Abbreviations used

IPTG: Isopropyl-β-D-thiogalactopyranoside

MS: Mass spectrometry

nSin a 2: Natural Sin a 2

rSin a 2: Recombinant Sin a 2 subunit

SPT: Skin prick test

The application of molecular biology techniques to the analysis of allergens can help to improve the knowledge of their structures, immunologic properties, and functions.²⁰

In addition, well-defined recombinant allergens might be used as diagnostic markers to characterize patients with allergies as well as to develop future therapeutic approaches.

Herein, we report the cloning and sequencing of a cDNA encoding a subunit of the 11S globulin Sin a 2 from yellow mustard seeds and its recombinant expression in *Escherichia coli*.

METHODS

Material

E. coli strain TOP10 and the plasmid pCR2.1, both included in the TOPO TA-cloning kit (Invitrogen, Groningen, The Netherlands), were used for cloning the polymerase chain reaction (PCR) products and sequencing. *E. coli* strain DH5α (Invitrogen, Carlsbad, Calif) was used as a host for plasmid pET-11b (Novagen, Darmstadt, Germany) to obtain the pET-11b/Sin a 2 construct. For expression of the recombinant protein *E. coli* strain BL21-CodonPlus (DE3) (Stratagene, La Jolla, Calif) was used. An antipolyhistidine monoclonal peroxidase conjugate antibody (Sigma-Aldrich, St Louis, Mo) was used to detect the recombinant protein. Yellow mustard seed extract and purified natural Sin a 2 (nSin a 2) were obtained as previously described.¹³

Cloning strategy of Sin a 2 subunit-cDNA

Total RNA from yellow mustard seeds was extracted as previously described²¹ and employed as a template to synthesize total cDNA by using the SMART RACE cDNA amplification kit (BD Biosciences-Clontech, Madrid, Spain). Oligonucleotides used in the PCR-based cloning are collected in Table I. Two degenerate oligonucleotides were used: a sense (SIN2-A) and an antisense (SIN2-B). An internal fragment of 580 base pairs was obtained, and its nucleotide sequence allowed the synthesis of 2 new nondegenerate oligonucleotides: SIN2-C and SIN2-D, which were used together with the nonspecific primer UPM to amplify the 5' and 3'

ends of the Sin a 2 subunit-encoding region, respectively. The full-length nucleotide sequence codifying for a Sin a 2 subunit (without the signal peptide) was amplified by using, in a single round of PCR, 2 new primers: a sense one, SIN2-E, and an antisense primer, SIN2-F. The signal peptide was deduced considering the data of the N-terminal sequence of the natural protein previously obtained by Edman degradation.¹³ All nucleotide sequencing was performed according to Sanger et al.²² Homology searches were performed using the BLAST program²³ and multiple amino acid sequence alignments using the CLUSTAL W program.²⁴

Bacterial expression of recombinant Sin a 2 subunit (rSin a 2)

The cDNA encoding Sin a 2 was amplified by PCR using the pCR2.1/Sin a 2 construct as template, 2 nondegenerate primers (sense primer S2-N, 5' cgcatagtGACAGTCGCTAGGGGTTCC 3' and an antisense primer S2-C, 5' cggaattcTCAGTGGTGGTGGTGGTGAACCTCGACTATCTCCTC 3') and the *taq*Gold DNA polymerase (Applied Biosystems, Foster City, Calif). The sense primer S2-N contains an *Nde*I restriction site (underlined above) and the sequence corresponding to amino acids at positions 1-7 (VQSLGVP) of the mature protein, in which the first amino acid (R in the native protein) was changed to V for ease of expression.²⁵ The antisense primer S2-C contains an *Eco*RI restriction site (underlined above), a stop codon, a sequence codifying for a His₆ tag, and the sequence corresponding to the last 6 residues of Sin a 2 (EEIVEV). The obtained fragment was cloned into the pCR2.1 for sequencing. This construct was digested with *Nde*I/*Eco*RI restriction enzymes, and the released fragment was subcloned into the same sites of the pET-11b vector under the control of the T7lac promoter. Afterward, *E. coli* BL21-CodonPlus (DE3) cells were transformed following the instructions provided by the manufacturer. Bacteria harbouring the expression vector were selected on LB agar plates containing chloramphenicol (50 μg/mL) and ampicillin (100 μg/mL). Several transformed clones were checked for recombinant protein expression. LB cultures were shaken at 250 rpm in an orbital incubator at 37°C until an A₆₀₀ = 1.0 was reached. The expression of the recombinant protein was induced with isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mmol/L. The cells were harvested after 2, 4, and 6 hours of induction, resuspended in a sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) sample solution containing 2-mercaptoethanol (1%), boiled at 95°C for 10 minutes, and used for immunoblotting detection.

Purification of rSin a 2

rSin a 2 was purified from bacterial lysates under denaturing conditions. Cells were harvested after 6 hours of IPTG induction, and the pellet was resuspended in 50 mL of binding buffer (Tris-HCl 100 mmol/L, NaCl 0.3 mol/L, tween-20 0.1%, urea 6 mol/L; pH 7.0).

TABLE II. Patient features with mustard allergy and sensitized to nSin a 2

Patient no.	Age/sex	Symptoms	Yellow mustard seeds		nSin a 2		rSin a 2
			Skin test*	ELISA (OD)†	Skin test*	ELISA (OD)†	ELISA (OD)†
1	72/F	OAS, AE	81	1.67	29	0.22	0.24
2	51/F	OAS, AE, U, GIS	110	3.37	69	0.98	0.73
3	51/M	OAS, AE	99	1.04	52	0.78	0.67
4	30/F	OAS, AE, CU, GIS	174	3.47	46	2.14	1.36
5	30/M	OAS, U, A	361	2.87	156	2.40	1.64
6	29/M	OAS, U, GIS, RC	54	2.81	37	0.46	0.33
7	36/M	OAS, AE, U	83	3.50	139	2.14	1.60
8	54/F	OAS, U, GIS, A, RC	43	0.85	35	0.53	0.39

F, Female; OAS, oral allergy syndrome; AE, angioedema; U, urticaria; GIS, gastrointestinal symptoms; M, male; CU, contact urticaria; A, asthma; RC, rhinoconjunctivitis.

*Wheal area in mm².

†Specific IgE determined in ELISA as OD at 492 nm.

Resuspended cells were disrupted by sonication in a digital sonifier over 30 seconds at an amplitude of 60%, followed by a resting time on ice over 30 seconds. This process was repeated 5 times. The lysate was centrifuged at 15,000g for 30 minutes at 4°C, and the supernatant was loaded onto a high-density IDA-agarose 6 BCL nickel charged column (Hispanagar, Madrid, Spain) equilibrated with the binding buffer. After washing with binding buffer, the proteins were eluted with a buffer composed of Tris-HCl 100 mmol/L, NaCl 0.3 mol/L, urea 6 mol/L, pH 7.0, and containing increasing concentrations of imidazole (10, 30, 200, and 1000 mmol/L). rSin a 2 eluted at 200-mmol/L imidazole. Fractions with rSin a 2 were pooled and ultrafiltered by using a centrprep YM-30 (Millipore, Billerica, Mass).

Analytical procedures

SDS-PAGE was performed according to Laemmli²⁶ in 15% (wt/vol) polyacrylamide gels, under nonreducing conditions. Proteins were visualized by Coomassie Brilliant Blue R-250 or alternatively transferred to nitrocellulose membranes (Amersham Biosciences, Buckinghamshire, United Kingdom). The protein concentration was determined using the method of Lowry et al²⁷ with bovine serum albumin (BSA) as standard. Digestion, mass spectrometry (MS), and MS/MS analyses of the excised bands from the SDS-PAGE separation of the mustard extract were carried out as previously described.¹³

Indirect enzyme-linked immunosorbent assay (ELISA), Western blot, and IgE immunoblot inhibition experiments

Quantification of IgE levels was performed by means of ELISA in microtiter plates coated with 20 µg/well of yellow mustard seeds extract or 0.2 µg/well of purified allergens, as described.²⁸ Briefly, plates were incubated with individual allergic sera (1:5 diluted). Binding of IgE was detected by mouse anti-human IgE antibodies (diluted 1:5000; donated by ALK-Abelló, Madrid, Spain) followed by horseradish peroxidase-labeled goat anti-mouse IgG (diluted 1:5000; Pierce Chemical Co, Rockford, Ill). Peroxidase reaction was developed using fresh enzyme substrate and measuring optical density (OD) at 492 nm. Each OD value was the mean of 2 determinations after blank subtraction. OD values <0.1 were considered negative responses.

Protein extracts, purified rSin a 2, or purified nSin a 2 were blotted onto nitrocellulose membranes after SDS-PAGE under nonreducing conditions. The membranes were incubated with patient sera (diluted 1:5) at 4°C overnight. Bound IgE antibodies were detected as described above. The peroxidase reaction was developed using the ECL Western blotting reagent (Amersham Biosciences).²⁹ The

recombinant protein was detected with the anti-polyhistidine monoclonal antibody (diluted 1:1000 in blocking buffer) at room temperature over 2 hours. The staining was developed as described.²¹

For inhibition Western blot assays, the pool of sera (diluted 1:5) or the individual sera (diluted 1:5) were alternatively preadsorbed to 10 µg of purified nSin a 2, to 10 µg of purified rSin a 2, or to 10 µg of BSA for control purposes, over 2 hours at room temperature, before the incubation of the corresponding membrane in each case. Volummograms of the reactive bands were analyzed by scanning densitometry using UVIBand V97 (UVItect St John's Innovation Center, Cambridge, United Kingdom).

Patients

To evaluate the IgE-binding capacity of rSin a 2 in comparison with nSin a 2, serum samples from 8 patients with mustard allergy were used.¹³ The selection criteria to include these 8 patients in the study was to have both positive specific IgE response and skin prick test (SPT) response to nSin a 2. Serum samples from patients with olive pollen allergy and persons without allergy were used as controls.

RESULTS

Characteristics of patients with mustard allergy

Patients with mustard allergy included in this study had a mean age of 44.1 years, and all of them had extraoral symptoms on eating mustard (Table II). Median value of SPTs was 83 mm² for mustard extract and 46 mm² for purified nSin a 2. Specific IgE antibodies for mustard extract, nSin a 2 and rSin a 2 are indicated as OD at 492 nm in Table II. A good correlation existed between the values for natural and recombinant allergens ($r = .99$, $P < .001$).

Molecular cloning and determination of the primary structure of Sin a 2 subunit

The nucleotide sequence of the Sin a 2 subunit cDNA (accession number AY846388 in the GenBank Database) as well as its deduced amino acid sequence is shown in Fig 1. The obtained cDNA comprises an open reading frame of 1530 bp that encodes a protein of 487 amino acids plus 23 residues of a signal peptide (Fig 1). The molecular mass and pI of Sin a 2 subunit, calculated from the deduced amino acid sequence excluding the signal

FIG 1. Nucleotide sequence of cDNA encoding Sin a 2 subunit and deduced amino acid sequence. Cleavage site for signal peptide is indicated by an arrow head. *Potential cleavage site to render the chains of the mature protein. Peptides identified in the natural allergen¹³ are underlined. Numerals on the right side indicate amino acid position in the mature protein.

Immunoblotting inhibition experiments were carried out for nSin a 2 and rSin a 2. When a pool of sera from patients with mustard allergy (patients 4, 6, 7, and 8) was preincubated with purified nSin a 2, the IgE-binding to

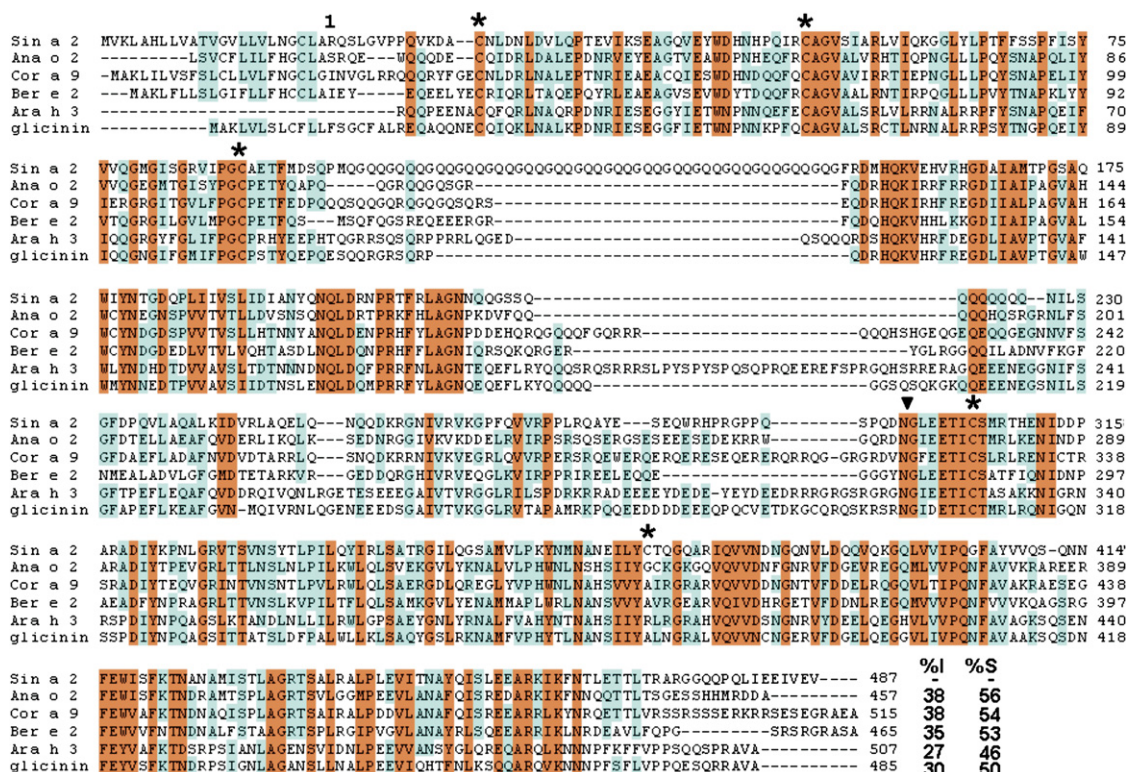


FIG 2. Alignment of sequences of allergenic 11S globulins. Yellow mustard seed (Sin a 2), cashew nut (Ana o 2), hazelnut (Cor a 9), Brazil nut (Ber e 2), peanut (Ara h 3), and soybean (glycinin) (GenBank accession numbers AY846388, AF453947, AF449424, AY221641, AF093541, and X15122, respectively). (-) for gaps. Residues conserved in all proteins are in orange, and those conserved in at least 4 chains are in blue. The Asn cleavage site is indicated by an arrow and cysteines by asterisks. %I and %S, percentages of identity and similarity, respectively. Amino acid numbering for Sin a 2 is given for the mature protein (R1-V487).

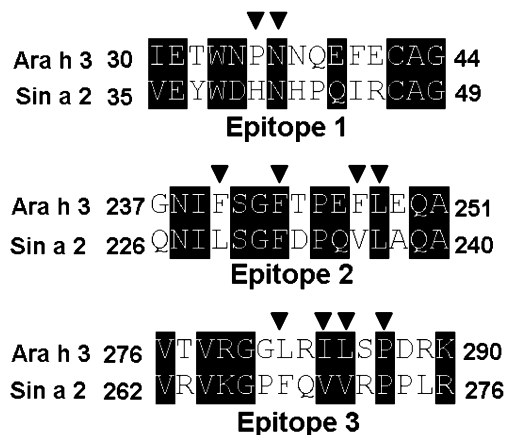


FIG 3. Comparison of peptides reported as epitopes in Ara h 3³⁰ with those corresponding to Sin a 2. Numerals indicate the position in each protein. Amino acids involved in the IgE binding in Ara h 3 epitopes are indicated by arrowheads. Conserved amino acids are shaded in black.

rSin a 2 was totally inhibited (Fig 5, B). When the same pool of sera was preincubated with rSin a 2, the IgE-binding to the 51 kD protein band of mustard extract was inhibited (Fig 5, C) as well as an IgE-reactive band of

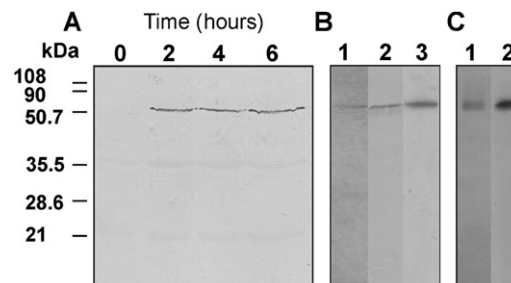


FIG 4. Analysis of the expression and purification of rSin a 2 by SDS-PAGE. **A**, Time course of the production; immunoblotting with anti-polyhistidine antibody. **B**, Purified rSin a 2 was stained with Coomassie Blue (lane 1), and immunostained with the anti-polyhistidine antibody (lane 2) and with a pool of sera from patients with mustard allergy (lane 3). **C**, Purified nSin a 2 was stained with Coomassie Blue (lane 1) and immunostained with the same pool of sera (lane 2). Molecular mass markers are displayed in the left margin.

about 32 kD. Moreover, the response to a 14 kD reactive band decreased. Protein bands at 32 and 14 kD were excised from the gel stained with Coomassie Blue (Fig 5, C, lane CBS) and analyzed by MS profile and MS/MS.

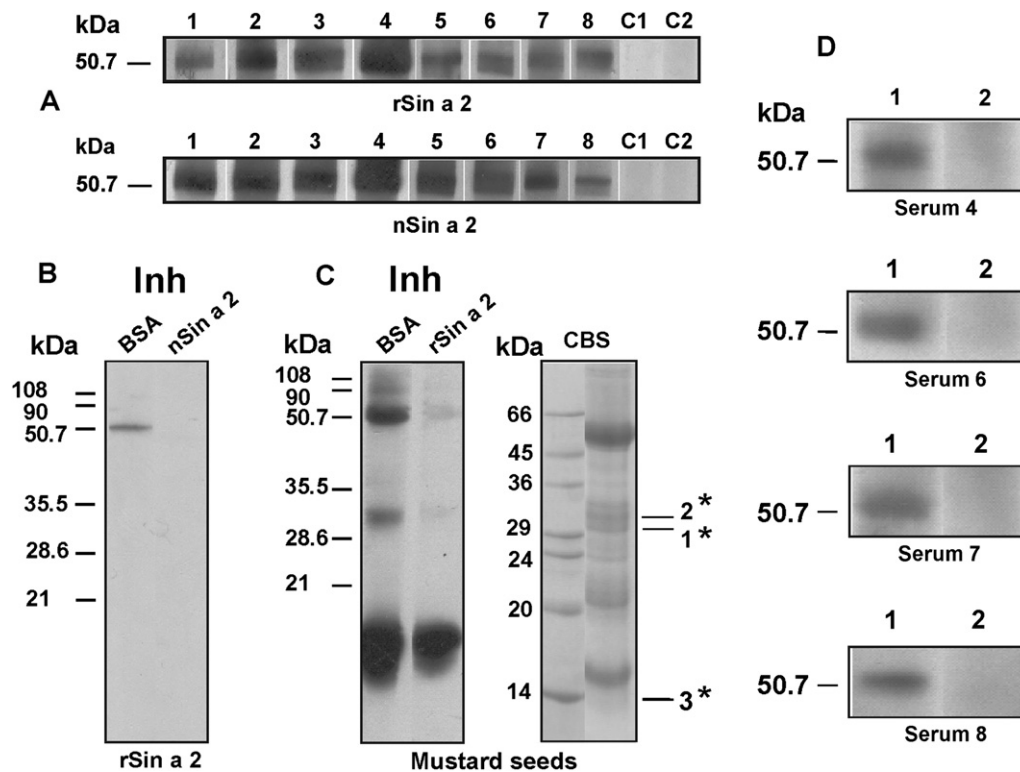


FIG 5. Immunodetection of rSin a 2 and inhibition assays after SDS-PAGE and transference to membranes. **A**, IgE responses to rSin a 2 and nSin a 2 with sera from 8 patients with mustard allergy (lanes 1 to 8); C1 and C2 are responses to sera from a patient with olive pollen allergy and from a person without allergy, respectively. **B**, IgE immunodetection of purified rSin a 2 with a pool of sera from patients 4, 6, 7, and 8 after preincubation with 10 μ g of BSA or with 10 μ g of nSin a 2. **C**, Yellow mustard seed extract (20 μ g total protein) was exposed to the same pool of sera preincubated with 10 μ g of BSA or 10 μ g of rSin a 2. Mustard seed extract (20 μ g total protein) was stained with Coomassie Blue (CBS). Asterisks indicate the protein bands analyzed by MS and MS/MS. **D**, IgE immunodetection of nSin a 2 after preincubation of the individual sera with BSA (lane 1) or rSin a 2 (lane 2). Molecular mass markers are in the left margin.

The combined search allowed the identification of 3 peptides from protein 1 that aligned with Sin a 2 subunit at positions A³⁷⁶-K³⁹⁵, T⁴²²-R⁴³⁴, and A⁴⁴⁰-E⁴⁸² (65%, 69%, and 56% identity, respectively). For protein 2, 3 peptides were identified and completely fitted with Sin a 2 at positions T³⁰⁸-R³⁴³, Y³⁶¹-K³⁹⁵, and I⁴⁶⁰-R⁴⁷¹. These results suggest that protein 2 might be a proteolytic fragment of the cloned Sin a 2 and that protein 1 might correspond to a fragment of a putative isoform. For protein 3, only peptides aligning with Sin a 1 were found, probably because it is the major component around 14-kD molecular mass. Finally, rSin a 2 inhibited approximately 87% (SD < 3%) of the IgE binding of the 4 individual sera to nSin a 2 (Fig 5, D).

DISCUSSION

In this work, we report the cDNA cloning, sequencing, and recombinant production of a subunit from Sin a 2, an 11S globulin that has been recently described as a major allergen in yellow mustard seeds.¹³ Two important goals have been approached in this work: (1) to know the Sin

a 2 sequence in order to deduce the structural properties of the molecule as well as to determine the identity degree with homologues in other foods; and (2) to compare the IgE-binding capability of the recombinant and natural allergens.

A PCR cloning strategy was designed based on the amino acid sequences obtained from peptides of the natural allergen.¹³ The nucleotide sequence of Sin a 2 subunit encodes a mature protein of 487 amino acids, which contains all peptides identified by Edman degradation and MS/MS in nSin a 2.¹³ This allergen does not contain any potential N-glycosylation site, which is according to the data published for 11S globulins.³¹

Sin a 2 was first described as an 11S legumin-type globulin as all identified peptides aligned with sequences of these proteins.¹³ The comparison of the whole sequence of Sin a 2 subunit with proteins from database banks has confirmed that it belongs to an 11S globulin. These proteins are hexameric oligomers, each subunit (approximately 50 kD) composed of 1 large acidic chain (30-40 kD) and another small basic chain (20 kD), which are bound by 1 disulfide bridge.³² Both chains come from a single polypeptide precursor, which is enzymatically

processed during the biosynthesis of the protein in the seeds. The cleavage of Sin a 2 subunit by the potential consensus site, located between positions Asn²⁹⁷ and Gly²⁹⁸, would split the allergen into 2 polypeptides: a large chain with a deduced molecular mass and pI of 32998 d and 7.93, respectively; and a small chain of 21200 d and 6.81, respectively. These data would be essentially in accordance with our previous results obtained for nSin a 2 (apparent molecular masses of 36 and 23 kd),¹³ as the small differences could be attributed to the defolding of the natural chains under reducing conditions used in the separation. Therefore, Sin a 2 represents, to our knowledge, the first 11S globulin displaying a large subunit with basic pI. In addition, a differential characteristic of Sin a 2 is the presence of 13 consecutive repetitions of the motif (Gly-Gln-Gln) at positions 109-147. The obtaining of the cDNA encoding a second isoform for the subunit of Sin a 2 would be in agreement with the fact that these proteins are encoded by multigene families, but it could also point toward a heteromeric composition of the hexamers. The detection of a protein band of about 32 kd that exhibited differences of sequence with the cloned Sin a 2 subunits also supports these observations.

Despite having low levels of sequence identity, 11S globulins present highly conserved 3-dimensional structures, and most of them share 2 independent beta-barrel domains so-called cupin domains.³³ The alignment of Sin a 2 with other allergens of the 11S globulin protein family showed an overall sequence identity under 40%, with Cor a 9 and Ana o 2 the molecules exhibiting the highest percentages of identity with Sin a 2. Although the relatively low levels of overall identity and similarity between Sin a 2 and Ara h 3 (27% and 46%, respectively), the comparison of the amino acids sequences of 3 peptides described as epitopes in Ara h 3,³⁰ with those positions corresponding to Sin a 2 revealed that some critical positions were conserved in both taxonomically unrelated allergens. Several authors have reported that patients with mustard allergy frequently display hypersensitivity to other vegetable foods, mainly nuts and legumes.^{9,34} 11S globulins from some of these biological sources have been described as allergens,¹⁴⁻¹⁸ but whether these molecules might be implicated in cross-reactivity processes or the observed association of hypersensitivity to mustard and other food sources is a consequence of cosensitization needs additional investigation.

In this work, we have achieved the recombinant production of a subunit from Sin a 2 in *E coli*. Because the protein lacked N-glycosylation sites and homologous globulins only displayed 1 disulfide bond, we judged this heterologous expression system as adequate for its recombinant production. The high correlation in the IgE-binding capacity of nSin a 2 and rSin a 2 demonstrated that rSin a 2 could be used to identify *in vitro* patients with mustard allergy with positive response to the 11S globulin. rSin a 2 was recognized in immunoblotting by the sera from all patients included in this study. These patients showed positive SPT against purified nSin a 2 (Table II), which indicates that this allergen can cause

release of inflammatory mediators in patients with mustard allergy. Differences observed in SPT results and specific IgE-binding values when using the whole mustard seed extract or nSin a 2 could be explained by the contribution of other important allergens such as Sin a 1. In addition, rSin a 2 inhibited the IgE binding to the natural allergen in immunoblotting experiments, which suggests that most IgE-binding epitopes contained in nSin a 2 are present in the recombinant allergen. Interestingly, when the pool of sera was preincubated with rSin a 2, not only the IgE binding to the 11S globulin was inhibited in yellow mustard seeds extract but also that detected at around 32 kd. From the MS analyses of these protein bands, it can be deduced that shorter components of mustard legumins, which probably resulted from proteolytic processing of the subunits, have also a relevant IgE-binding potential. This fact has also been demonstrated for vicilin from pea,³⁵ and it may occur in some species.³¹

In summary, the availability of rSin a 2 opens new perspectives of research directed to resolve whether 11S globulins might be implicated in cross-reactivity among taxonomically nonrelated allergenic sources and could contribute to detect the presence of specific IgE in sera from patients allergic to mustard.

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