ORIGINAL ARTICLE

Association Between the Seed Storage Proteins 2S Albumin and 11S Globulin and Severe Allergic Reaction After Flaxseed Intake

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Abstract

Background: Given the increased popularity of flaxseed in meals, several cases of allergy to these seeds have been reported. Little is known about the allergens implicated in hypersensitivity reactions to flaxseed. The present study aimed to identify the allergens involved in IgE-mediated reactions in 5 patients with a clinical history of severe systemic symptoms after flaxseed consumption.
Methods: Proteins that were potential allergens with IgE-binding capacity were purified from flaxseed extract using chromatography and identified via MALDI-TOF mass spectrometry. Immunoassays were performed using the 5 allergic patients' sera tested individually and as a pool.
Results: Immunoblotting of the flaxseed extract revealed a low-molecular-mass protein (around 13 kDa) in 4 of the 5 patients, while a protein of approximately 55 kDa was detected in 2 patients. The proteins were identified by mass spectrometry as flaxseed 2S albumin, which is included in the WHO/IUIS allergen nomenclature as Lin u 1, and 11S globulin. Inhibition assays revealed in vitro IgE-mediated cross-reactivity between Lin u 1 and peanut and cashew nut proteins, while IgE-mediated recognition of 11S globulin by patients' sera was partially inhibited by several plant-derived sources.
Conclusions: Seed storage proteins from flaxseed were involved in the development of severe symptoms in the 5 patients studied and exhibited cross-reactivity with other allergenic sources. Besides the severity of flaxseed allergy in patients sensitized to 2S albumin, this is the first time that 11S globulin has been identified as a potential allergen. Taking these data into account should ensure a more accurate diagnosis.

Key words: Food allergy. Flaxseed. 2S albumin. 11S globulin. Cross-reactivity. Anaphylaxis.

Resumen

Antecedentes: Dada la creciente popularidad de la linaza en las comidas, se han notificado varios casos de alergia a estas semillas. La información acerca de los alérgenos implicados en las reacciones de hipersensibilidad a estas semillas es escasa. El presente trabajo pretende identificar los alérgenos implicados en las reacciones mediadas por IgE en cinco pacientes con una historia clínica de síntomas sistémicos graves tras el consumo de linaza.
Métodos: Las proteínas susceptibles de ser alérgenos con capacidad de unir IgE se purificaron a partir del extracto de linaza mediante técnicas cromatográficas. Su identificación se realizó mediante espectrometría de masas MALDI-TOF. Se realizaron inmunoensayos con los sueros de los cinco pacientes alérgicos, utilizados de forma individual o como mezclas.
Resultados: Cuatro de los cinco pacientes reconocieron una proteína de baja masa molecular (alrededor de 13 kDa) en inmunoensayos con extracto de linaza, mientras que dos pacientes reconocieron una proteína de aproximadamente 55 kDa. Se identificaron por espectrometría de masas como albúmina 2S de linaza, incluida en la nomenclatura de alérgenos de la OMS/IUIS como Lin u 1, y globulina 11S, respectivamente. Los ensayos de inhibición *in vitro* revelaron la existencia de reactividad cruzada de la Lin u 1 con las proteínas del cacahuete y del anacardo, mientras que el reconocimiento por parte de la IgE de la globulina 11S por parte de los sueros de los pacientes fue parcialmente inhibido por varias fuentes vegetales.
Conclusiones: Las proteínas de almacenamiento de las semillas de lino estaban implicadas en el desarrollo de síntomas graves en cinco individuos y mostraron una reactividad cruzada con otras fuentes alergénicas. Además de la gravedad de la alergia a la linaza en los pacientes sensibilizados a la albúmina 2S, es la primera vez que se identifica la globulina 11S como un alérgeno potencial. Consideramos que estos datos deben ser tenidos en cuenta para un diagnóstico más preciso de los pacientes.

Palabras clave: Alergia alimentaria. Linaza. Albúmina 2S. Globulina 11S. Reactividad cruzada. Anafilaxia.

Introduction

In recent years, edible seeds from plants such as sesame, sunflower, and pumpkin have been incorporated into the human diet owing to their putative beneficial properties [1]. However, this has led to the appearance of hypersensitivity reactions to these foods that very often involve severe allergic symptoms. The identification of the proteins that act as elicitors of reactions remains a considerable challenge for specialists, since the allergens are generally present in small amounts or as hidden components in many food formulas [2].

Among seeds recently incorporated into the diet, *Linum usitatissimum* also known as flaxseed or linseed, is considered a "superfood" because of its content in protein, dietary fiber, polyunsaturated fatty acids, and lignin. Some of these compounds have been reported to have nutritional benefits, such as anti-inflammatory, antioxidant, and cardioprotective properties [3], and some flaxseed proteins have emulsifying capacity, which is very useful in the food technology industry [4]. Lately, flaxseed has been widely consumed because of its laxative effect [5,6]. Consequently, cases of food allergy have increased after intake, with severe reactions such as anaphylaxis.

Few cases of hypersensitivity reactions to flaxseed have been described. In 2002, Leon et al [7] first reported a patient sensitized to a 56-kDa protein from flaxseed, pointing out malate dehydrogenase (MDH) as an allergen. Two other case reports of flaxseed hypersensitivity [8,9] involved protein bands of around 22 and 20 kDa in the sensitization of 2 patients, and a potential lipid transfer protein (LTP) was indirectly involved in a patient with anaphylaxis to linseed [10]. A 2S albumin cDNA and its genomic sequence were identified from flaxseed and named as conlinin by Truska et al [11] in 2003. However, it was not until 2018 that the potential implication of this cDNA in flaxseed allergy was reported [12].

The aim of the present study was to identify and characterize the allergens involved in the severe allergic reactions experienced by 5 patients after consumption of flaxseed. We detected a 2S albumin and an 11S globulin. Based on the patients' clinical histories, we also evaluated the implication of these allergens in cross-reactivity with other seeds and nuts containing homologous proteins.

Materials and Methods

Patients' Sera

We recruited 5 well-characterized patients with a clear history of immediate systemic reactions after ingestion of flaxseed, as follows: 3 from the Allergology Department of Hospital Universitario Fundación Jiménez Díaz, 1 from Hospital Clínico San Carlos III, and 1 from Hospital de la Princesa, all in Madrid, Spain. The 5 individuals had positive skin prick test (SPT) and/or specific IgE (ImmunoCAP, ThermoFisher) results for flaxseed extract following the diagnostic algorithm of the European Academy of Allergy and Clinical Immunology [13]. The study was approved by the local ethics committees (PIC28/2014) and written informed consent was obtained from all the patients.

Linseed skin tests were carried out using the prick-by-prick technique. The size of the reaction was recorded as a mean wheal diameter (D+d/2), with D indicating the larger diameter of the wheal and d indicating the largest diameter orthogonal to D. A prick test with a response at least 3 mm greater in diameter than the saline control was considered positive.

The study fulfilled the Ethics Guidelines of Complutense University of Madrid.

Preparation of Protein Extracts From Plant-Derived Sources and Purification of Seed Storage Proteins

Delipidated protein extracts were obtained from various vegetable sources, flaxseed included, as previously described [14]. Protein concentrations were determined using the Lowry method.

Seed storage proteins from flaxseed were first isolated using size exclusion chromatography with a Sephadex G-50 medium column equilibrated with 0.15 M ammonium bicarbonate pH 8.0 at a flow rate of 3 mL/min. The resulting fractions were analyzed using SDS-PAGE. The low-molecular-mass protein fractions were pooled and underwent a second step in reversed-phase high-performance liquid chromatography (RP-HPLC) using a C-18 reversed-phase column, with an acetonitrile gradient of 0% to 60% at a flow rate of 1.5 mL/min. Fractions were analyzed again by SDS-PAGE and stained with Coomassie blue R-250 (Sigma-Aldrich). The cashew nut allergen Ana o 3 was isolated following this methodology, while the peanut allergen Ara h 2 was obtained from the manufacturer (Indoor Biotechnologies).

High-molecular-mass proteins were isolated using anionic exchange chromatography with a DEAE-Cellulose matrix and a gradient of 0.02 M to 0.5 M ammonium bicarbonate pH 8.0 at a flow rate of 1 mL/min, followed by a second superfine size exclusion chromatography procedure. The fractions obtained were examined using SDS-PAGE, and the bands were visualized using Coomassie blue R-250 staining. The mustard allergen Sin a 2 was also isolated using this method.

Identification of 2S Albumin and 11S Globulin by Mass Spectrometry

The identity of the purified low-molecular-mass protein was evaluated using matrix-assisted laser desorption/ionization (MALDI)-time of flight (TOF) mass spectrometry. For this purpose, SDS-PAGE was run under "sterile" conditions (buffers prepared in sterile Milli-Q water) after isolation of the proteins, and the gel was stained with colloidal Coomassie blue G-250. Stained bands were excised and conserved in ultrapure Milli-Q water for peptide mass fingerprinting in the Proteomics Department at the Complutense University of Madrid, Spain. Samples were analyzed in a mass spectrometer (4800 Proteomics Analyzer, AB SCIEX). This spectrometer has a MALDI ionization source and 2 TOF analyzers in tandem, thus providing us with the molecular masses of proteins and their fingerprint patterns. For high molecular-mass protein, apart from its fingerprint determination, 4 peptides were obtained by de novo synthesis.

Analytical Procedures for Protein Characterization

Purity and apparent molecular masses were monitored using SDS-PAGE in 15% polyacrylamide gel (for high-

molecular-mass proteins) or 17% polyacrylamide gel (for low-molecular-mass proteins). Samples were diluted in loading buffer (50 mM Tris-HCl, pH 6.8; 2% [wt/vol] SDS, 10% [wt/vol] glycerol, 2 mM EDTA, 0.01% [wt/vol] bromophenol blue) in the presence or absence of the reducing agent β-mercaptoethanol (βME, 5% of sample volume) and were denatured for 10 minutes at 90ºC. Coomassie blue R-250 was used for staining. Molecular mass was determined using protein markers (Thermo Fisher). The 2S albumin concentration was determined by measuring absorbance at 280 nm (E0.1% = 1.28 µL/µg·cm), while the 11S globulin concentration was obtained using the bicinchoninic acid (BCA) method (BCA Pierce, Thermo Fisher).

Western Blotting and Inhibition Assays

Purified proteins (2 µg) and food extracts (20 µg) were blotted onto nitrocellulose membranes (Amersham Biosciences) after SDS-PAGE under nonreducing conditions (–βME). Immunodetection of potential allergenic proteins was performed as previously described [15] using individual sera or a pool of sera diluted 1:5 in blocking solution (3% skim milk powder) in phosphate-buffered saline (PBS: 8.1 mM Na2HPO4, 1.5 mM KH2PO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4) containing 0.1% Tween-20 (PBS-T buffer) at a final volume of 300 µL per lane of extract or isolated protein. Nonatopic serum was used as a negative control. Human IgE binding was detected using mouse antihuman IgE monoclonal antibody (1:5000 diluted [ALK-Abelló]), followed by horseradish peroxidase–labeled polyclonal rabbit antimouse IgG (1:2000 diluted [Pierce]). The chemiluminescent signal was developed using ECL-Western blot reagent (Clarity Western ECL Substrate, Bio-Rad) and detected in an LAS3000 luminescent image analyzer (FujiFilm). The signal was quantified in triplicate using Multigauge V3.0.

For the immunoblotting inhibition assays, equivolumetric pools of patients' sera diluted 1:5 in blocking buffer were preincubated with 20 µg of the corresponding extracts (0.1 mg/mL inhibitor) or 5 µg of purified allergens (0.025 mg/mL inhibitor) using BSA as a negative control at room temperature for 2 hours before incubation with the membranes. The subsequent steps were as described above.

ELISA Assays

Indirect ELISA was performed in 96-well polystyrene plates (3950 high-binding plates, Costar) coated with purified proteins (2 µg/mL in PBS, pH 7.5, 100 µL/well) and incubated overnight at 4ºC. The plates were washed with PBS-T and blocked with 3% skimmed milk powder in PBS-T, followed by incubation with patients' sera diluted 1:5 for 2 hours at 37ºC. The plates were washed with PBS-T and incubated with mouse antihuman IgE antibody, followed by horseradish peroxidase–labeled goat antimouse IgG antibody, both for 1 hour at 37ºC, with PBS-T washes after each incubation. IgE binding was detected using o-phenylenediamine and H_2O_2 in 0.1 M citrate buffer. The reaction was stopped with the addition of 3N H_2SO_4. The absorbance in each well was measured at 492 nm in an iMark Microplate Absorbance Reader (Bio-Rad), and nonspecific binding of antibodies was eliminated by subtracting the absorbance for control wells containing no antigen from the absorbance for antigen-coated wells. Positive reactions were considered those with absorbance measurements at 492 nm greater than 3-fold the standard deviations above the mean. A nonatopic serum was used as a negative control.

Results

Immunological Detection of Potential Allergens From Flaxseed Extract in Patients' Sera

The patients' general characteristics are shown in the Table. All patients reported immediate allergic reactions after intake of flaxseed as part of a snack or meal. These involved severe and systemic reactions, such as anaphylaxis, with positive skin prick test (SPT) results for flaxseed extract in patients #2 to #5. All of them exhibited different sIgE levels to flaxseed extract, with the lowest value recorded for patient #1 (0.85 kU/L), whose serum recognized only the high-molecular-

Table 1. Patients' Clinical Information

			SPT[a]	ImmunoCAP[b]							
Patient no.	Age/ Sex	Symptoms	Flaxseed	Flaxseed	Mustard seed	Peanut	Cashew nut	Sesame seed	Pru p 3	Pollen allergy	Other food allergies
1	44/M	AN	ND	0.86	15.00	ND	ND	ND	ND	Yes	m
2	44/M	D, G, OAS	7.5	10.50	ND	1.90	ND	0.30	1.33	Yes	al, h, p, s, soy, w, wa, wm
3	38/F	AN	11.5	4.59	Neg	Neg	Neg	Neg	Neg	Yes	Neg
4	41/F	AN	22	3.17	ND	ND	Neg	ND	Neg	Yes	sf
5	9/F	AN	11	7.23	ND	ND	ND	ND	ND	Yes	e

Abbreviations: al, almond; AN, anaphylaxis; D, diarrhea; e, egg; F, female; G, gastrointestinal symptoms; h, hazelnut; OAS, oral allergy syndrome; m, mustard seed; M, male; ND, no determined; Neg, negative (wheal mean diameter <7 mm for SPT); p, pistachio; Pos, positive (wheal mean diameter >7 mm for SPT); s, sesame seed; sf, seafood; soy, soya; SPT, skin prick test; wa, walnut; wm, watermelon w, wheat.
[a]Mean diameter, mm.
[b]Specific IgE, kU_A/L.

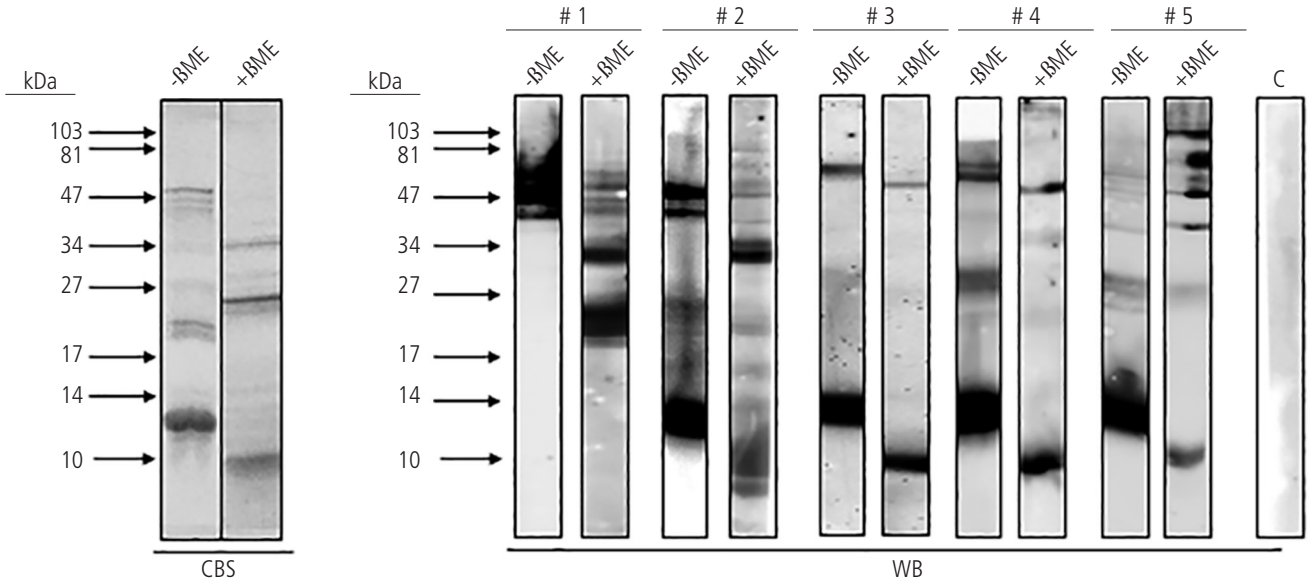


Figure 1. IgE-mediated recognition of proteins on flaxseed extract. SDS-PAGE and Coomassie blue staining (CBS) of flaxseed extract. Western blotting (WB) of flaxseed extract tested with sera from 5 patients under nonreducing (–βME) and reducing (+βME) conditions. A nonatopic serum was used as a negative control. C indicates control.

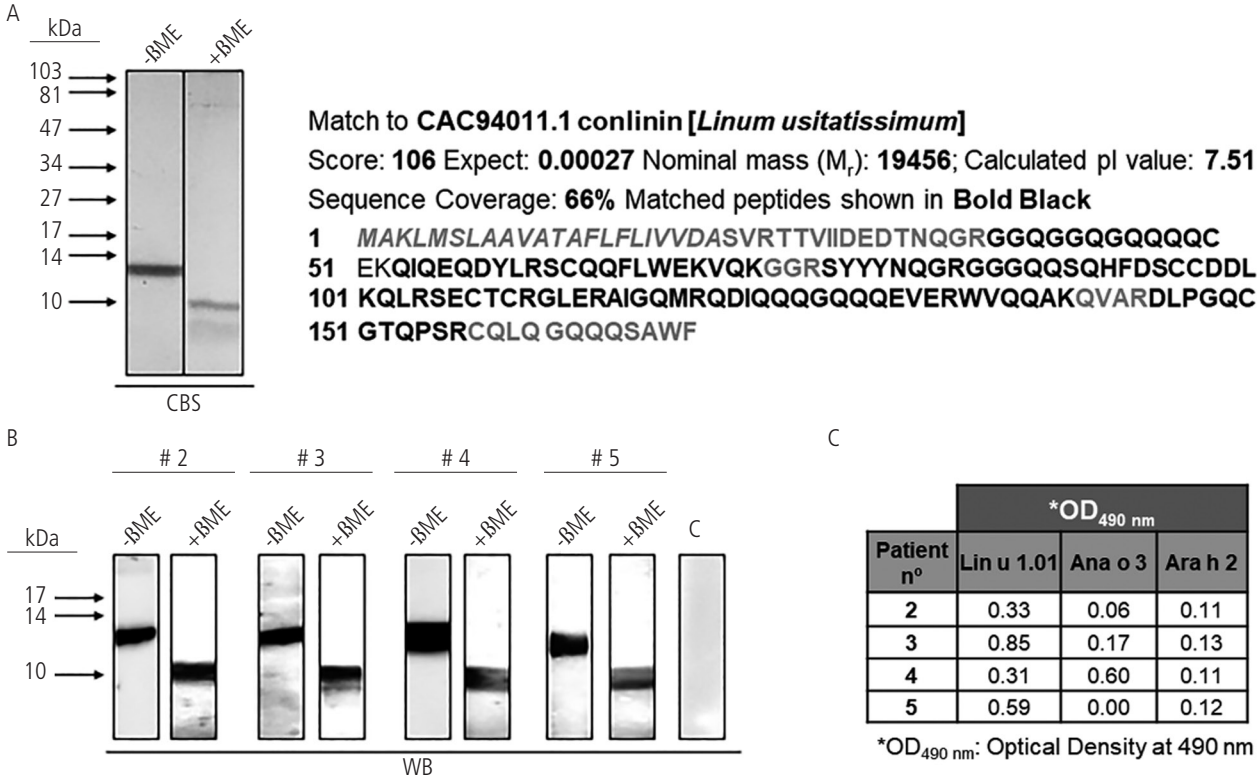
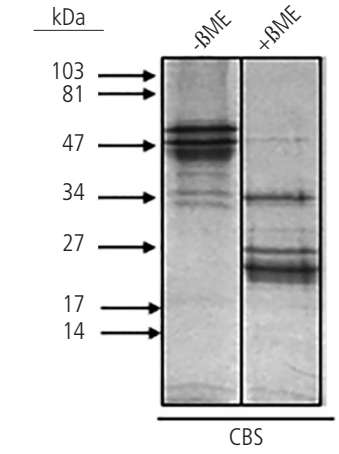


Figure 2. Identification and immunodetection of flaxseed 2S albumin. A, SDS-PAGE of purified flaxseed 2S albumin with and without βME. The signal peptide determined using the SignalP program is shown in italics; fingerprint-matched peptides with conlinin are shown in bold. This protein has been included in the WHO-IUIS database as an allergen (Lin u 1.01). B, IgE-mediated recognition by Western blot (WB) of isolated 2S albumin from flaxseed by serum #2, #3, #4, and #5 under nonreducing (–βME) and reducing (+βME) conditions. C, ELISA of purified 2S albumin from flaxseed (Lin u 1), cashew nut (Ana o 3), and peanut (Ara h 2) with individual serum from flaxseed-allergic patients. Nonatopic serum was used as a negative control (ELISA: OD490 nm ≤0.1). C indicates control; CBS, Coomassie blue staining.

A



De novo sequence	Protein match	Accesion number
ALPADVLANA*WR* or **ALPADVLANA*VS*R** **QSSERFEWVAFK**	11S globulin isoform 3 [*Castanea sativa*]	**AGE34644**
EVSIFSPGNQSQQR	11S globulin [*Pistacia vera*]	**ABU42022**
FFLAGNPQR or ***K*FFLAGNPQR**	11S globulin subunit beta [*Nicotiana attenuata*]	**A0A1J6L9S7**
FTETQEQQPQR	legumin-related high molecular weight polypeptide [*Vicia faba var. minor*]	**CAA81261**

B

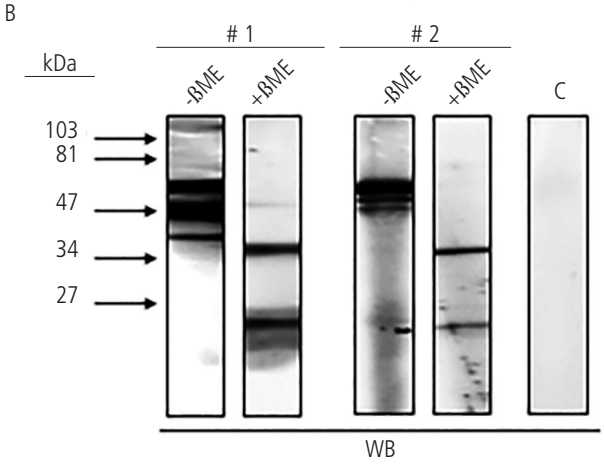


Figure 3. Identification and immunodetection of flaxseed 11S globulin. A, SDS-PAGE and identification of flaxseed 11S globulin by de novo sequencing. Possible variations in amino acid sequences are shown in bold italics. The peptides obtained matched with 11S globulins from several plant sources. B, Isolated 11S globulin recognition by sera #1 and #2, under nonreducing (–βME) and reducing (+βME) conditions. A nonatopic serum was used as a negative control. C indicates control; CBS, Coomassie blue staining.

mass protein, and values ranging from 3.2 to 10.5 kU/L for the other 4 patients.

In order to determine the proteins involved in allergic reactions against flaxseed, patients' sera were tested by immunoblotting with flaxseed extract in the presence and in the absence of the reducing agent βME. Reducing conditions disrupted the disulphide bonds that maintain the heterodimeric structure of many storage protein families present in the seeds; these are visible in the changes in electrophoretic mobility of the extract. Immunoassays (–βME) revealed 2 IgE-binding bands with an apparent molecular mass of 13 and 55 kDa. Four of the 5 patients presented IgE-mediated reactivity to the 13-kDa IgE-binding band, which dropped to a band of 10 kDa when the reducing agent was added. Serum #1 only recognized a ~55 kDa protein (–βME) that seemed to split into a 34-kDa band and a 22-kDa band (+βME), probably corresponding to polypeptide chains of the 55-kDa protein; serum #2 recognized both proteins from the flaxseed extract (Figure 1).

Isolation, Identification, and IgE-Mediated Recognition of Flaxseed 2S Albumin and 11S Globulin

Recognition of IgE to flaxseed extract in patients' sera was investigated by isolating immune-reactive proteins using different chromatographic methods (Figure 1 Supplementary). Coomassie blue staining after electrophoresis confirmed that the 13-kDa protein split into 2 subunits of 10 kDa and ~3 kDa, which were heavy and light polypeptide chains, respectively (Figure 2A), while the 55-kDa protein showed the 34-kDa and 22-kDa subunits, as in the extract (Figure 3A).

In order to identify the nature of these proteins and the families they belonged to, mass-spectrometry analyses were carried out with purified proteins. The low-molecular-mass protein was identified as flaxseed 2S albumin (previously known as conlinin according to the UniProt database) by peptide mapping, and its fingerprint was obtained by MALDI-TOF/TOF techniques, with 66% coverage (Figure 2A). A 55-kDa

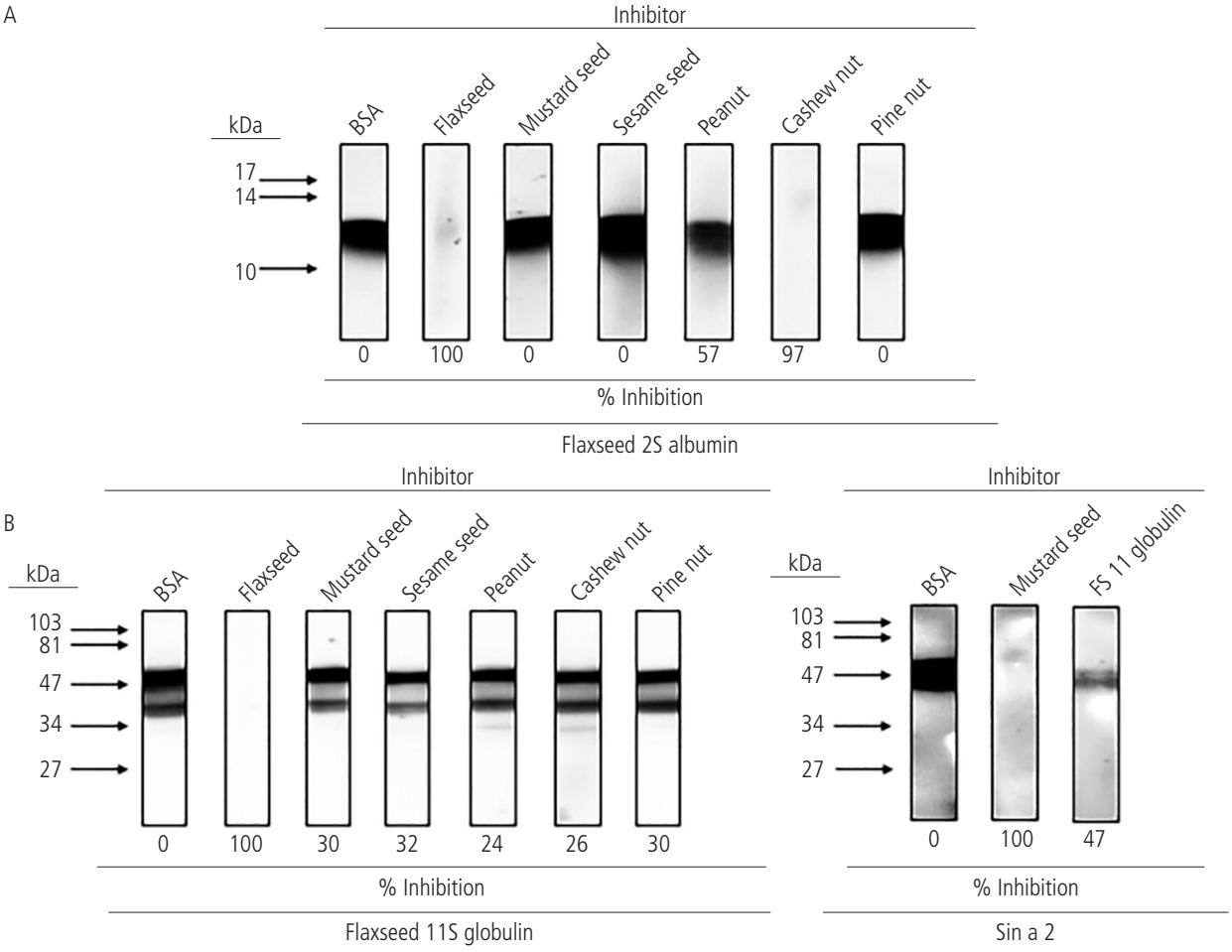


Figure 4. Immunoblotting inhibition assays (–βME). A, Inhibition of recognition of purified flaxseed 2S albumin (2 µg) by IgE using different extracts (200 µg; 100 µg/mL) as inhibitors, with pooling of patients' sera (#2, #3, #4 and #5). B, Inhibition of recognition of purified 11S globulins from flaxseed and mustard seed (2 µg) by IgE using different extracts (200 µg; 100 µg/mL) and proteins (5 µg; 25 µg/mL) as inhibitors, with pooling of patients' sera (#1 and #2).

flaxseed protein required de novo sequencing procedures for identification, yielding 4 peptides that matched with 11S globulins from different vegetable species (Figure 3A).

Flaxseed 2S albumin was recognized by 4 out of 5 allergic patients' sera by Western blot and ELISA (Figure 2B and C). Under reducing conditions, patient sera reacted exclusively against the 10-kDa heavy chain of the 2S albumin. Sera from patients #1 and #2 recognized flaxseed 11S globulin, as well as its 2 subunits, under reducing conditions (Figure 3B).

In Vitro Cross-Reactivity of Flaxseed Storage Proteins With Other Plant-Derived Food Sources

Inhibition assays were conducted to determine the potential role of the flaxseed allergens in cross-reactivity with other food sources. Thus, recognition of IgE to the 2S albumin Lin u 1 by a pool of patients' sera (#2, #3, #4, #5) was completely blocked by cashew nut extract (97%) and partially inhibited by peanut (57%). No inhibition was obtained with pine nut or mustard and sesame seeds, although patient #2 was sensitized to sesame and developed clinical symptoms after sesame seed intake (Figure 4A).

IgE binding to the 11S globulin was partially blocked when a mixture of 2 sera from patients #1 and #2 that recognized the 11S globulin was incubated with extracts from mustard seed (30%) and sesame seed (32%), as well as peanut (24%), cashew nut (26%), and pine nut (30%) (Figure 4B). The cross-reactivity exhibited by this 11S globulin might explain the severe allergy symptoms to yellow mustard seed in patient #1 and to sesame in patient #2, as their medical histories revealed. An equivolumetric mixture of IgEs from these 2 patients was able to recognize mustard seed 11S globulin (Sin a 2), and the purified flaxseed 11S globulin was able to partially inhibit this reactivity (47%) (Figure 4B). The data indicate possible cross-reactivity between flaxseed and yellow mustard seed due to their 11S globulins. Inhibition immunoblotting showed that flaxseed and mustard seed inhibited recognition of each other's 11S globulin.

Discussion

Seeds commonly induce allergic systemic reactions including anaphylaxis. Incorporation of novel ingredients, such as edible

seeds from vegetable foods (eg, sunflower, pumpkin, flax, or sesame seeds) into our habitual diet and other products has led to the description of new cases of hypersensitivity reactions to these compounds when consumed as a food, in cosmetics through contact, or as laxative drugs, as in the case of flaxseed [16]. We report 5 cases of food allergy to flaxseed in which the patient developed systemic symptoms. Despite reports of hypersensitivity reactions to these seeds [17], little information is available on the molecular protein components involved.

The immune profiles resulting from testing patients' sera with flaxseed extract revealed 2 potential allergens. The 13-kDa protein, which was recognized by most individuals' sera, was identified as a 2S albumin whose cDNA had previously been sequenced and named as conlinin [11]. This component was recently associated with flaxseed allergy [12]. Nevertheless, the low number of patients involved in that study and the lack of biochemical data were insufficient to enable conlinin to be considered a relevant allergen. The present study provides information about the structure of these allergens and more evidence on the severity of the reactions to flaxseed in patients sensitized to 2S albumins, as occurs with other proteins from the same family [18]. The data provided in the present study led conlinin to be included as allergen Lin u 1.01 in the official allergen database maintained by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee.

Regarding IgE-binding profiles, Lin u 1 displayed similar features to allergens from other vegetable sources, namely, a heterodimer with 2 polypeptide heavy and light chains linked by disulphide bonds [19]. 2S albumins are characterized by a hypervariable region located in the heavy chain and containing the most relevant epitopes involved in food allergy [20].

The low similarity between the amino acid sequences of 2S albumins has made it difficult to predict their potential cross-reactivity (Figure 2 Supplementary), which is usually restricted to proteins from the same phylogenetic family [14,21]. Despite the low conservation of amino acid sequences, 2S albumins present a highly preserved 3D structure [22]. Therefore, possible similarities at the 3D level (structural epitopes) may be involved in the cross-reactivity observed between flaxseed, cashew nut, and peanut through their 2S albumins. Most patients are sensitized to multiple plant-derived foods (eg, mustard seed, tree nut, sesame seed). However, here we report in vitro cross-reactivity only between flaxseed and cashew nut or peanut mediated by their respective 2S albumins. The link between peanut and cashew nut and flaxseed was already reported at the clinical level [23], although no data have been reported at the molecular level to date. This is not the first time we have reported cross-reactivity between unrelated sources mediated by their 2S albumins [24,19]. These allergens have usually been associated with primary sensitization, as described for Sin a 1 from mustard seed [25]. The current study provides valuable information for diagnosis, since little is known about the cross-reactive potential of these allergens.

Sera from patients #1 and #2 also recognized a protein of around 55 kDa, which proteomics-based analysis revealed to be the 11S globulin of flaxseed. The small number of patients prevented us from including this protein as an allergen in the IUIS database; however, inhibition assays showed the great potential of this protein in cross-reactivity with several plant-derived sources, as previously observed [26]. A larger cohort of patients is needed to determine the relevance of this protein in allergy to flaxseed.

In conclusion, we describe 2 IgE-binding storage proteins, a 2S albumin and an 11S globulin, which are involved in severe allergic reactions after flaxseed consumption. The 11S globulin showed broader and partial cross-reactivity with other plant-derived sources (seeds and nuts) than that displayed by 2S albumin. Interestingly, this is the first report of a 2S albumin cross-reacting with a 2S albumin of cashew nut, a finding that warrants further evaluation. Identification of potential allergens from edible seeds is a considerable challenge for specialists, since these seeds, which are part of complex foods and snacks, act as a hidden allergenic source. However, while interesting, our data should be corroborated in a higher number of flaxseed-allergic patients. The description of these 2 flaxseed IgE-binding proteins and their cross reactivity will help not only to improve the diagnosis and treatment of allergic patients, but also to resolve the complex issue of cross-reactivity between seeds and nuts in food allergy. Appropriate management of allergic reactions to food can reduce health care costs and improve patient quality of life.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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