

Identification and Characterisation of the IgE-Binding Proteins 2S Albumin and Conglutin γ in Almond (*Prunus dulcis*) Seeds

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Key Words

Almond seed · 2S albumin · Conglutin γ · IgE binding · Cross-reactivity

Abstract

Background: Almond proteins can cause severe anaphylactic reactions in susceptible individuals. The aim of this study was the identification of IgE-binding proteins in almonds and the characterisation of these proteins by N-terminal sequencing. **Methods:** Five sera were selected from individuals with a positive reaction to food challenge. Sodium dodecylsulphate-polyacrylamide gel electrophoresis and immunoblotting were performed on almond seed proteins. Purified IgE-binding proteins were tested for immunoblot inhibition with sera pre-incubated with extracts of hazelnut and walnut. **Results:** N-terminal sequences of the 12-, 30- and 45-kD proteins were obtained. The 45- and 30-kD proteins shared the same N terminus, with 60% homology to the conglutin γ heavy chain from lupine seed (*Lupinus albus*) and to basic 7S globulin from soybean (*Glycine max*). The sequences of the N-terminal 12-kD protein and of an internal peptide obtained by endoproteinase digestion showed good homology to 2S albumin from English wal-

nut (Jug r 1). Immunoblot inhibition experiments were performed and IgE binding to almond 2S albumin and conglutin γ was detected in the presence of cross-reacting walnut or hazelnut antigens. **Conclusions:** Two IgE-binding almond proteins were N-terminally sequenced and identified as almond 2S albumin and conglutin γ . Localisation and conservation of IgE binding in a 6-kD peptide obtained by endoproteinase digestion of 2S albumin was shown.

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Introduction

Food allergens are one of the major causes of hypersensitivity in susceptible individuals. The allergies are usually linked with the synthesis of specific IgE antibodies directed against one or several food proteins. IgE antibodies bind allergens in limited protein portions, the antigenic determinants. These epitopes may be linear, conformational or a combination of the two. IgE-mediated reactions include eczema and gastro-intestinal symptoms (vomiting, diarrhoea and abdominal cramps) in infants and oral allergy syndrome, urticaria/angio-oedema, rhinoconjunctivitis or anaphylaxis in adults. Food allergies are

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potentially associated with life-threatening, IgE-mediated systemic reactions in some individuals. In recent years, an increase in tree nut and peanut allergies has been reported in Europe and in the US [1–5]. The acquisition of multiple allergies is often caused by progressive sensitisation during childhood due to exposure to foods containing similar antigens. Sensitisation to other fruits of the Rosaceae family [6, 7] or to peanut, which is a member of the legume family, has been reported. In the US, peanut and/or tree nut allergy affects approximately 1.1% of the population, corresponding to 3 million individuals at risk of adverse reactions to these foods. Of these individuals, 50% of those considered in the survey performed by Sicherer et al. [5] were reactive to peanut, 30% to walnut and 10% to almond, while only 4% were reactive to both peanut and tree nuts. In several reports, the percentage of allergic individuals symptomatically reactive to two or more nuts (English walnut, almond or hazelnut) has been found to be nearly 10%; this corresponds to at least half a million individuals in the world reactive to two related nuts. Almonds, like other nuts, are increasingly consumed and often used as a hidden ingredient, thus making avoidance especially difficult for allergic patients. To date, few studies on almond allergy have been reported. In one recent study, IgE-mediated allergy to almonds was found in 22% of symptomatic patients by means of positive skin prick tests and ImmunoCAP® with IgE antibodies specific to almond [8]. Another recent report on the identification of almond allergens was based on a single case allergy [9], but only one major IgE-binding almond protein has been characterised as an allergen, namely almond major protein or amandin [10]. In this present work, two IgE-binding proteins isolated from almond seeds are reported and identified by sequence homology as 2S albumin and conglutin γ proteins.

Materials and Methods

Plant Material

Seeds of *Prunus dulcis* (Mill.) D.A. Webb, syn. *P. amygdalus* Batsch, and *P. communis* (L.) (cultivar Tuono) were collected at late stages of maturation in August to September.

Acetone Powder Preparation

Almond extract was prepared using a low-temperature acetone powder method. Mature seeds (400 g) were homogenised twice with 10 volumes of diethylether (Sigma Diagnostics, St. Louis, Mo., USA) for 30 s at -20°C in a Waring blender at maximum speed. The homogenate was separated by filtration and washed with 10 volumes of cold acetone. The powder was dried at room temperature and stored at -20°C .

Protein Extraction and Purification

Total proteins were extracted from 20 g of acetone powder with 10 volumes of 10 mM K-phosphate buffer (PBS), pH 7.4, containing 150 mM NaCl, 0.5 mM phenylmethylsulphonylfluoride and 2 mM β -mercaptoethanol. Samples were homogenised in a Waring blender for 2 min at 4°C and centrifuged at 10,000 g for 30 min at 4°C . The supernatant was adjusted to pH 5 and centrifuged to remove insoluble globulins. The clear supernatant was brought to pH 7, and solid ammonium sulphate was added to the combined supernatants at 40% saturation. The precipitated proteins were centrifuged and resuspended in 1–2 ml of 25 mM Tris-hydroxymethylaminomethane (TRIS) buffer, pH 7.5. The proteins were desalted in 25 mM TRIS buffer, pH 7.5, by passing them through a Sephadex G-100 (Amersham Pharmacia Biotech, Little Chalfont, UK) column. The fraction, eluted as a single peak, was subjected to ion exchange chromatography on a MonoQ column (Pharmacia, Uppsala, Sweden), pre-equilibrated with 25 mM TRIS buffer, pH 7.5, using a Biosys 510 Protein Purification System (Beckman, Fullerton, Calif., USA), and fractions (1 ml) were collected. The unbound proteins were collected by elution from the ion exchange column with the same buffer. The bound proteins were eluted with a three-step gradient (100–200, 200–300 and 300–500 mM) of NaCl in 25 mM TRIS buffer, pH 7.5. Collected fractions were separated by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) in 10–12% acrylamide. Proteins were quantified by the method of Bradford [11] using bovine serum albumin as a standard. Purified 12-kD protein was digested by agarose-bound Glu-endoproteinase (Sigma Diagnostics). The obtained peptide fractions were separated by gel filtration with a P6 Sephadex column (Pharmacia) and by SDS-PAGE. To separate low-molecular-weight peptides at high resolution, SDS-PAGE in 16% acrylamide with a discontinuous TRIS-tricine buffer system was performed [12].

Immunoblot Analysis

Almond proteins separated by SDS-PAGE were tested by immunoblotting analysis using a standard procedure [13]. Proteins were transferred to nitrocellulose membrane and incubated for 1 h in a blocking solution (3% bovine serum albumin in PBS containing 0.1% Tween 20; PBS-T) and then for 1 h with selected sera diluted 1/200 in PBS-T, followed by 3 washes with PBS-T. Blotted membranes were then incubated with anti-IgE antibodies (Sigma) conjugated to horseradish peroxidase, diluted 1/1,500 in PBS-T, and signals were detected by chemiluminescence using an ECL kit (Amersham Pharmacia Biotech). Immunoblots were carried out with the 5 unpooled sera from individuals with a positive double-blind, placebo-controlled food challenge (DBPCFC) and with one serum with a positive radio-allergosorbent test (RAST) analysis, showing a highly reproducible pattern of IgE binding. The sera were tested in the immunoblot analysis individually. In a few experiments performed in the final steps of large-scale purification of 12- and 45-kD proteins, two sera (1 and 2) with high IgE content were used pooled together. A serum from a non-allergic individual was used as a negative control.

Immunoblot Inhibition Tests

Purified almond proteins were blotted onto membranes and tested with sera pre-incubated for 2 h with hazelnut and English walnut extracts (1.5–5 mg of total proteins or 0.25–0.5 mg of albumin fraction in 1 ml of serum diluted 1/200 in PBS-T). Of the six sera already tested, it was found sufficient to carry out cross-inhibition tests for only two.

Peptide Sequencing

Amino-terminal sequence analysis of the 12- and 45-kD proteins was performed with a Pulse liquid-phase protein sequencer (477A, Applied Biosystems-Perkin Elmer, Foster City, Calif., USA). Proteins separated by SDS-PAGE were electroblotted onto polyvinylidene difluoride (PVDF) membrane (Amersham Pharmacia Biotech). The blotted polypeptides, stained with 0.1% Coomassie brilliant blue G-250 (Sigma Diagnostics) in 50% methanol and 10% acetic acid, were excised from the membrane and arranged into the sequencer cartridge. Phenylthiohydantoin amino acids from Edman degradation phases were identified by HPLC with a phenylthiohydantoin analyser (ABI-Perkin Elmer Model 120A) in line with the sequencer. To screen for sequence homology, the SwissProt Data Library was used. Two peptide fractions after Glu-C endoproteinase digestion (6- and 2-kD fractions) were separated on a Nucleosil-ODS C 18 column by reverse-phase (RP) chromatography. Peptides purified by RP-HPLC were sequenced on a Procise 492 instrument (Applied Biosystems).

Patients and Sera

Sera from individuals with a demonstrated reactivity to almond or other nuts were obtained from two different Immunology and Allergy Centres (the Allergology Centre at the Niguarda Ca' Granda Hospital, Milan, and the Allergology Department, Fazzi Hospital, Lecce, Italy). In the Lecce area, birch pollen allergy is rare and patients present mainly with food allergies. Sera from patients allergic to nuts or legumes were selected by means of positive skin prick tests and the appearance of oral allergy symptoms stage 4 (anaphylactic reaction in patients 1–4) or oral allergy syndrome stage 2 (itching of oral cavity and rhino-conjunctivitis in patient 5). Skin prick tests with a commercially available almond extract were positive for the selected sera, with histamine as positive and saline solution as negative controls. The concentration of total IgE was measured by the RAST method. Sera were selected on the basis of positive RAST values for sera positive for English walnut, hazelnut, soybean and/or peanut, with values scoring above 5 being included in classes II and III. Sera were characterised for the presence of specific IgE antibodies reactive to English walnut, hazelnut, peanut and/or soybean allergens. The concentration of specific IgE was measured using the Pharmacia CAP System [14]. Of the observed values, scores superior to 0.35 were considered positive. DBPCFC tests or open food challenges were performed on the selected individuals at the Allergology Centre in Milan. Five of the tested sera were positive to food challenges and negative to placebo (patients 1–4 had an anaphylactic reaction and patient 5 had oral cavity itching). A sixth serum, provided by the Allergology Department in Lecce, was selected only on the basis of the RAST positive reaction, without further analyses. By testing this serum in the immunoblot analysis, an IgE-binding profile similar to that obtained with the sera of patients positive to DBPCFC was found.

Results

Immunoblot Analysis and Separation of IgE-Binding Proteins

To identify the IgE-reactive proteins, immunoblot experiments with almond extracts were performed using the sera from 5 individuals positive to skin prick tests and

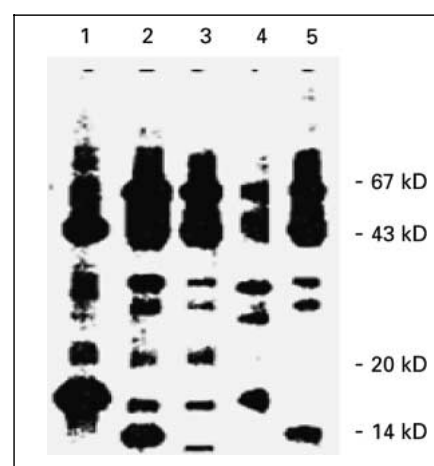


Fig. 1. Immunoblot analysis of almond proteins. Total extracts were separated by SDS-PAGE in an 8–15% acrylamide gradient. Lanes 1–5: sera from individuals positive to DBPCFCs.

open food challenges and from 1 individual positive to the RAST test, as described in Materials and Methods. The 5 sera reacted to almond proteins from total extracts with similar protein bands, and therefore the identified IgE-binding proteins were considered putative almond allergens (fig. 1). Immunoblots with these sera revealed an IgE-binding profile ranging in relative molecular weight from 12 to 100 kD. The most abundant bands present in almond extract ranged from 12 to 66 kD. IgE-binding almond proteins were purified by means of a three-step purification process: globulin separation, ammonium sulphate precipitation and anion exchange chromatography. The globulins were separated by precipitation at pH 5, the albumin fraction was fractionated by 40% ammonium sulphate saturation and the precipitated proteins were desalted by gel filtration on a Sephadex G-100 column. The desalted proteins were separated by HPLC using a MonoQ column (fig. 2a); four main peaks were obtained using a three-step gradient of NaCl. Each fraction was analysed by SDS-PAGE and Western blotting. As shown in figure 2c, the immunoblot analysis revealed two major IgE-binding proteins with molecular weights of 12 and 45 kD (lanes U and B2, respectively).

Sequencing of the 45-kD Almond Protein

Single protein bands were sequenced as described in Materials and Methods. N-terminal amino acid sequencing of almond proteins was successful for three different bands. The N-terminal amino acid sequence of the 45-kD

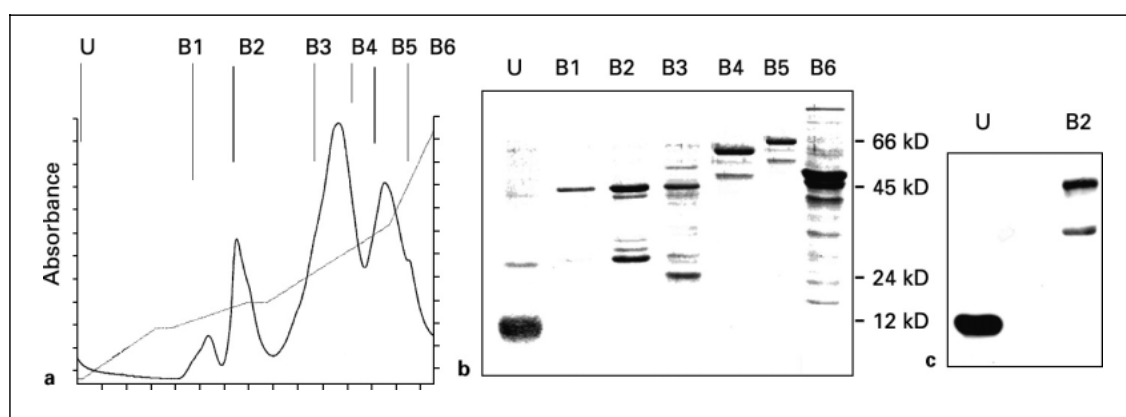


Fig. 2. Purification and immunoblot analysis of almond protein extracts. a MonoQ chromatography profile of the almond albumin fraction obtained after precipitation with 40% ammonium sulphate saturation as described in Materials and Methods. b SDS-PAGE of protein fractions eluted from MonoQ chromatography (U: unbound; B1–B6: bound fractions); proteins were detected by Coomassie brilliant blue staining. c Immunoblot analysis after transfer to nitrocellulose membrane and incubation with selected serum (number 3), with IgE binding to the 12-kD (U) and 45-kD proteins (B2).

Table 1. Alignment of the N-terminal amino acid sequence of the 45-kD almond protein with the mature form of 45-kD conglutin γ heavy chain from white (CAC17729) and narrow-leaved blue lupine (S21426), conglutin-like protein from *Arabidopsis* (Q9ZVS4) and basic 7S globulin from soybean (AAB03390)

Protein	N-terminal amino acid sequence
Almond 45-kD conglutin γ	T K S Q T H V P I R P N K L V L K V Q K D R A T N
Lupine conglutin γ	Y H N S Q P T S G S K P N L L V L P I Q Q D A S T K
<i>Arabidopsis</i> 45-kD protein	F S L S S A Q T P F R P K A L L L P V T K D Q S T L
Soybean basic 7S globulin	F F L S D S V T P T K P I N L V L P V Q N D G S T G

Letters in bold show amino acid identity or similarity.

protein was determined and compared with published sequences. On the basis of the 26 amino acids identified (table 1), the best alignment was obtained by comparing the N-terminal sequences of the 45-kD almond protein and lupine seed conglutin γ [15]. A sequence identity of 40% and homology of 60% were found with the 45-kD mature form of conglutin γ from white and narrow-leaved blue lupine. High similarity (50%) was also found to basic 7S globulin from soybean [16] and to a conglutin-like protein from *Arabidopsis*. On the basis of the homology with the mature conglutin γ in white lupine and other conglutins (molecular weight and high similarity of the N-terminal amino acid sequence), the sequence of the 45-kD IgE-binding protein was registered in the SwissProt database

with the accession number P82952 as almond conglutin γ protein.

The sequence analysis of a 30-kD protein, separated by MonoQ chromatography (fraction B2) and blotted onto a PVDF membrane, produced an N-terminal sequence identical to that obtained from the 45-kD conglutin γ . These data show the localisation of IgE-binding epitopes within the 30-kD N-terminal region of the almond conglutin γ protein. As seed conglutins are processed in two subunits, an N-terminal 28- to 30-kD heavy-chain subunit and a C-terminal 17-kD small subunit [15], it is suggested that the sequenced 30-kD almond peptide corresponds to the heavy-chain subunit originating from the conglutin γ protein.

Table 2. Alignment of the N-terminal amino acid sequence of the 12-kD almond protein with the mature form of a different 2S albumin

Protein	N-terminal amino acid sequence
Almond 12-kD protein	V T X E E G X Y S I S D Q S K V G E
English walnut 2S albumin	Q N L N H C Q Y Y L R Q Q S R S G Q
Brazil nut	Q Q L S H C R M Y M R Q Q M E E S P
Peanut	V N L K P C E Q H I M Q R I M G E
Castor bean	Q N L R Q C Q E Y I K Q Q V S G Q
Soybean	V N L T P C E K H L M E K I Q G R

The high similarity is shared with 2S albumin from English walnut, the allergen Jug r 1. One undetermined amino acid (X) could correspond to cysteine. Letters in bold show amino acid identity or similarity.

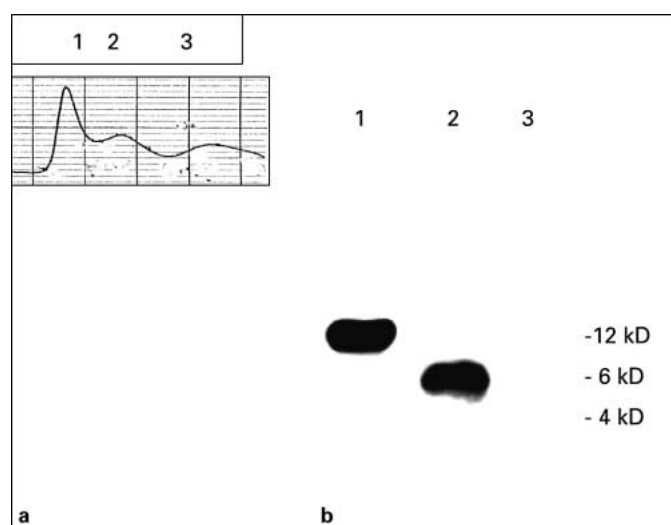


Fig. 3. Western blot analysis of peptide fractions obtained by endoproteinase digestion of the 12-kD almond protein. Blotted protein was incubated with selected sera (number 1 pooled with number 2) as described in Materials and Methods. Inset: gel filtration chromatography profile of the reaction products obtained by endoproteinase digestion of the 12-kD protein. Lane 1: undigested 12-kD protein; lane 2: 6-kD fraction; lane 3: 2-kD fraction.

Sequencing of the 12-kD Almond Protein

Subsequently, the sequence of the 12-kD IgE-binding protein was determined. Comparison of 18 N-terminal amino acids (table 2) with sequences recorded in the SwissProt database showed no identity with other database proteins. However, similarity with a sequence in 2S albumin from English walnut was observed (table 2). To confirm this similarity, the 12-kD protein was digested with Glu-endoproteinase and, after separation of the peptides by gel filtration chromatography, a major peptide

Table 3. Alignment of the N-terminal amino acid sequence of the 2-kD peptide obtained by cleavage of the 12-kD almond protein with sequences located near the C terminal of 2S albumins

Protein	N-terminal amino acid sequence
Almond 2-kD protein	Q X I R S P D R E M
Walnut	E C G I S S Q R C E I
Brazil nut	R C N L S P Q R C P M
Soybean	Q C D L S S D D
Peanut	Q C N F G A P Q R C D

High similarity (80%) between almond and English and Brazil nut 2S albumin was observed. One undetermined amino acid (X) could correspond to cysteine. Letters in bold show amino acid identity or similarity.

fraction of 6 kD and a second fraction in the 2-kD range were obtained. Immunoblots were performed to test the IgE reactivity of the SDS-PAGE-separated peptides. The immunoblot analysis showed that only the 6-kD peptide fraction conserved IgE-binding activity (fig. 3). The 6-kD peptide fraction was separated by RP-HPLC and showed the presence of three components. After the separation of the major fraction, attempts to perform amino acid sequencing were unsuccessful, probably because of the blocked N terminal. The 2-kD fraction was separated by RP-HPLC and one 2-kD peptide was sequenced successfully. This sequence shows 80% similarity to sequences near the C terminal of English walnut and Brazil nut 2S albumins (table 3). From these data and from the high methionine content detected in the sequence analysis data of the blotted 6-kD peptide fraction, it is very likely that the 12-kD almond protein belongs to the sulphhydryl-rich 2S albumin family of seed storage proteins [17–20]. On the basis of the overall similarity between the 12-kD

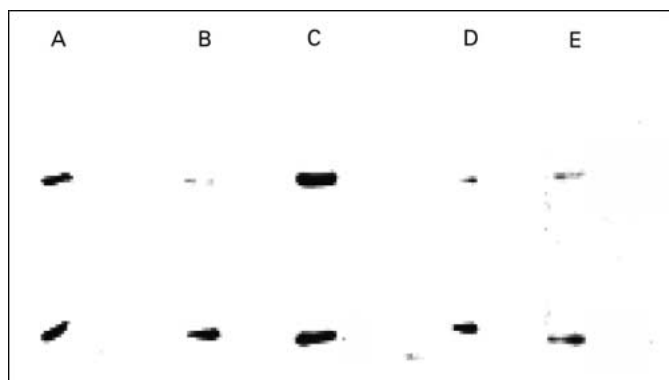


Fig. 4. Immunoblot inhibition experiments. Sera were pre-incubated with hazelnut and walnut albumin fractions, before Western blot analysis of 12- and 45-kD almond proteins transferred to nitrocellulose membranes. A: immunoblot analysis with serum (number 5) pre-incubated with 250 µg of walnut proteins; B: immunoblot analysis with serum (number 3) pre-incubated with 500 µg of walnut proteins; C: immunoblot analysis with control serum; D: immunoblot analysis with serum (number 5) pre-incubated with 250 µg of hazelnut proteins; E: immunoblot analysis with serum (number 3) pre-incubated with 500 µg of hazelnut proteins.

almond protein and 2S albumins (similar molecular weight and the high degree of similarity in the C-terminal amino acid sequence of 2S albumin in English walnut), the sequences from this IgE-binding almond protein were registered in the SwissProt database, under the accession number P82944, as almond 2S albumin.

IgE Cross-Reactivity to Other Nuts and Immunoblot Inhibition

To analyse the cross-reactivity of the IgE antibodies in the tested sera with proteins of other related nuts, total protein extracts and the globulin fractions of hazelnut and English walnut were separated by SDS-PAGE, and immunoblots were performed with one of the tested sera. Several protein bands were detected by IgE-binding analysis (data not shown). Hazelnut and walnut proteins, either as total extracts or as albumin fractions, were utilised for the immunoblot inhibition experiments. Sera were incubated with TBS buffer together with hazelnut or walnut proteins (1.5–5 mg of total extracts or 0.25 and 0.5 mg of soluble albumin fractions) before performing the analysis of IgE binding to membranes blotted with 45- and 12-kD almond proteins. In the immunoblot inhibition experiments using the nut albumin fractions, IgE binding to conglutin γ was visibly inhibited by the soluble fractions. Free IgE antibodies detected almond 2S albumin more

intensely (fig. 4). The different cross-reactivity of the soluble protein fraction compared to the total extract could have been caused partly by a higher content of IgE-binding proteins in the total extract. A decrease in IgE binding to almond proteins was observed in both the sera tested, i.e. that pre-incubated with hazelnut and that pre-incubated with walnut total extract. However, when the time of chemiluminescence reaction was extended, immunodetection of almond 2S albumin and conglutin γ by specific IgE antibodies was observed.

Discussion

The availability of several sera from individuals with allergies to almond and other nuts who were reactive to skin prick tests and positively responsive to almond in oral food challenge tests allowed the isolation of two different IgE-binding almond proteins. By means of chromatography separation, SDS-PAGE and protein blotting, the N-terminal sequence of three purified proteins and peptides led to the identification of two IgE-binding proteins in almond seed. This is the first report on the identification of 2S albumin and conglutin γ proteins in almond, and on their characterisation as IgE-binding proteins. The analysis of IgE binding to two different peptides obtained either by protease cleavage, as in the case of protein processing, or by endoproteinase digestion experiments, led to a broad localisation of IgE-binding domains in the 30-kD heavy chain of almond conglutin γ and in a 6-kD N-proximal peptide in almond 2S albumin. Many plant proteins in the 2S albumin family have been identified as major allergens, for example in English walnut [17], Brazil nut [18], sunflower [19] and castor bean [20]. The finding that almond 2S albumin exhibits high IgE-binding activity suggests that it could be a member of the 2S albumin allergen family present in seed [21]. However, the IgE-binding and serological reactivity of these proteins does not imply the clinical symptoms of the allergy; tests of clinical reactivity, such as open food challenges or DBPCFCs, will be needed to confirm this. Of the 7S seed storage proteins, only the peanut small conglutin subunit (17.5-kD light chain) Ara h 2 has been identified as a major allergen. To date, no conglutin γ heavy chain with IgE-binding activity has been reported. Basic 7S globulin from soybean, although having an amino acid composition similar to conglutin γ proteins, has never been reported as being involved in IgE binding as a soybean allergen. The finding of IgE reactivity in conglutin γ proteins implies the need for further investigation. In studies

on lupine allergies, it has been shown that one of the major allergens in lupine seed flour is a 43-kD seed storage protein [22]. Due to the similarity in molecular weight and the N-terminal sequence homology of almond and lupine conglutin γ proteins, the 43-kD IgE-binding lupine protein found in previous reports could correspond to lupine conglutin γ . Studies should be performed to identify the IgE-binding epitopes in almond conglutin γ , to test lupine conglutin γ for IgE binding and to verify the clinical reactivity of these proteins using DBPCFC tests. The addition of lupine flour to foods is a common practice. The hidden presence of lupine flour in foods could be a potential cause of cross-reaction or sensitisation in nut-allergic patients, especially in young children.

This study shows the presence of hazelnut and walnut proteins competing for IgE binding with almond 2S albumin and conglutin γ . Immunological cross-reactivity is common between members of the same botanical family. Several immunochemical methods (blots, ELISA, affinity chromatography) have been developed for the enrichment of specific antibodies and their application to the detection of tree nut allergens. Sandwich blots have been developed for antibody purification by stripping sera with fixed cross-reacting proteins or by absorption to bound antigens. In studies on hazelnut proteins, immunoblot detection by IgE antibodies in the sera from allergic individuals has been shown to be non-specific to hazelnut proteins [23]. After IgE separation by a sandwich ELISA test, no substantial cross-reactivity with other nuts, legumes or other food constituents was observed. Thus, a system has been developed for the effective detection of hazelnut traces in several consumer products. In studies with almonds [24], partially purified polyclonal antibodies from immunised sheep were used to develop an ELISA assay for the detection of almond traces in foods consid-

ered to be free of hazelnuts. These antibodies, specific to almonds, retained partial cross-reactivity with some tree nuts (black walnut, hazelnut, brazil nut). A similar approach was used by Roux et al. [10] for the detection of the almond major protein allergen in foods. In a study by Eigenmann et al. [25], the specificity of IgE binding in peanut was analysed using a preliminary absorption to soybean proteins of cross-reacting antibodies. After this pre-treatment, soy-adsorbed sera contained IgE antibodies specific to soybean, and the remaining non-adsorbed IgEs identified unique peanut allergens. In this study, hazelnut and walnut extracts were used for immunoblot inhibition experiments to block cross-reacting IgE antibodies by means of IgE binding to the antigens present in these related nut species. A decrease in total IgE binding with conservation of specific IgE binding to almond 2S albumin and conglutin γ was observed. This result shows the feasibility of IgE separation methods based on IgE binding to cross-reacting antigens blotted onto membranes or cross-linked to sepharose. By means of this methodology, the development of immunochemical methods with purified almond-specific IgEs could prove to be useful for the highly sensitive detection of almond allergens in food samples.

Almond 2S albumin and conglutin γ protein have been submitted to the SwissProt database under the accession numbers P82944 and P82952.

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