

Vicilin and convicilin are potential major allergens from pea

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Summary

Background Allergic reactions to pea (*Pisum sativum*) ingestion are frequently associated with lentil allergy in the Spanish population. Vicilin have been described as a major lentil allergen.

Objective To identify the main IgE binding components from pea seeds and to study their potential cross-reactivity with lentil vicilin.

Methods A serum pool or individual sera from 18 patients with pea allergy were used to detect IgE binding proteins from pea seeds by immunodetection and immunoblot inhibition assays. Protein preparations enriched in pea vicilin were obtained by gel filtration chromatography followed by reverse-phase high-performance liquid chromatography (HPLC). IgE binding components were identified by means of N-terminal amino acid sequencing. Complete cDNAs encoding pea vicilin were isolated by PCR, using primers based on the amino acid sequence of the reactive proteins.

Results IgE immunodetection of crude pea extracts revealed that convicilin (63 kDa), as well as vicilin (44 kDa) and one of its proteolytic fragments (32 kDa), reacted with more than 50% of the individual sera tested. Additional proteolytic subunits of vicilin (36, 16 and 13 kDa) bound IgE from approximately 20% of the sera. The lentil vicilin allergen Len c 1 strongly inhibited the IgE binding to all components mentioned above. The characterization of cDNA clones encoding pea vicilin has allowed the deduction of its complete amino acid sequence (90% of sequence identity to Len c 1), as well as those of its reactive proteolytic processed subunits.

Conclusions Vicilin and convicilin are potential major allergens from pea seeds. Furthermore, proteolytic fragments from vicilin are also relevant IgE binding pea components. All these proteins cross-react with the major lentil allergen Len c 1.

Keywords food allergy, legume cross-reactions, pea allergy, *Pisum sativum*, vicilin

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Introduction

Allergic reactions provoked by the ingestion of legume seed-derived foods, particularly peanut, soybean, lentil, chickpea and pea, are common [1–5]. However, sharp differences in the legume species mainly consumed, as well as in their clinical prevalence and cross-reactivity, are observed in different geographic areas. Thus, peanut and soybean are the major species involved in IgE-mediated hypersensitivity responses in countries such as United States, United Kingdom and Japan [2, 6]. Adverse reaction to other legumes, and true clinical cross-reactivity between peanut and other legumes (i.e. soybean), have been rarely reported [7]. In contrast, lentil, chickpea and pea are the main offending legumes in the Mediterranean area and in several Asian countries, like India [3–5]. Convincing clinical symptoms to lentils have been reported in up to 10% of patients with food allergy of the Spanish paediatric population [3]. Furthermore, approximately 70% of these patients show associated reactions to pea

and/or chickpea, as supported by positive open food challenges with the three legumes [8]. However, the molecular basis of such clinical cross-reactions had not been investigated, probably because the relevant allergens of these foods, except for lentil, remain unknown.

Vicilin (Len c 1) and its proteolytic subunits have been identified as major lentil allergens [9, 10]. Members of the same protein family are also important allergenic components in peanut (Ara h 1) and soybean, as well as in other plant seeds [11, 12]. Vicilins, also named 7S globulins, are major storage proteins present in most legume seeds. They are trimeric oligomers with no interchain disulphide bonds, formed by the *in vivo* association of mature monomeric chains ranging from 40 to 70 kDa. Extensive proteolytic processing and/or glycosylation may occur in some species, such as pea, rendering a wide spectrum of subunits (fragments) with different sizes, which usually remain associated with the mature chains [12, 13]. Similarity among vicilins from different species, both at the amino acid sequential and three-dimensional structural level, could lead to cross-reactivity. This seems to be the recently described case of three Dutch patients with anaphylaxis to pea, which subsequently showed peanut allergy [14].

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Taking into account the scenario described above, we have studied the potential relevance of vicilin and its subunits as pea allergens in a group of 18 pea-allergic Spanish patients, most of who were also sensitized to lentil. The reactive proteins were identified by means of N-terminal amino acid sequencing and cDNA cloning. Moreover, cross-reactive IgE to pea and lentil vicilins was shown in this group of patients.

Materials and methods

Patients and sera

Eighteen patients with adverse reactions after pea ingestion were selected from a population with allergy to legumes during a prospective study carried out by the Servicio de Alergia of Hospital Universitario La Paz (Madrid, Spain). The selection was based on: (i) a convincing clinical history of pea allergy, confirmed by positive open food challenge responses to this legume; (ii) high specific serum IgE levels (>3.90 kU/L) to pea, and (iii) positive skin prick test (SPT) responses to pea. All patients avoided all legumes in their diet after diagnosis. Informed consent was obtained from all participants. Clinical and demographic data of selected patients are summarized in Table 1. All patients, except patient no. 16, were sensitized to other inhalant and/or food allergen sources, besides legumes. These sources were mainly pollens (grass and/or olive; $n = 12$), fish ($n = 6$) and egg ($n = 5$).

IgE levels were determined by means of the CAP-FEIA System (Pharmacia Diagnostic, Uppsala, Sweden). SPTs were performed with a crude pea extract, by using standard methods [15].

A serum pool or individual sera from each of the selected patients were used in immunodetection and immunoblot inhibition assays. A pool of sera from five patients with allergy to fish but not to pollens or plant foods was tested as a negative control.

Pea extracts

Mature pea seeds (*Pisum sativum* cultivar Rommel) were ground, defatted with cold acetone ($2 \times 1:10$ w/v; 1 h; 4°C), dried and extracted with phosphate-buffered saline (PBS) buffer (0.1 M Na phosphate, 0.15 M NaCl, pH 7.4; $1 \times 1:5$ w/v; 1 h; 4°C). After centrifugation ($16\,300 \times g$; 30 min; 4°C), the supernatant was dialysed (cut-off point 3.5 kDa) against distilled H_2O and freeze-dried. Pea boiled extract was similarly obtained, but seeds were previously boiled for 30 min in water and then freeze-dried.

Protein content was quantified according to Bradford [16].

Protein preparations enriched in pea vicilins

The crude pea extract was redissolved in 0.5 M NaCl, and precipitated with ammonium sulphate at 85% saturation (2 h at 4°C). After centrifugation ($16\,300 \times g$; 30 min; 4°C), the supernatant (enriched in vicilins) was dialysed against H_2O , freeze-dried and then fractionated by gel-filtration chromatography on a Sephacryl S-200 column (2.5×90 cm; Amersham Biosciences, Piscataway, NJ, USA), eluting with 3 M urea in 0.1 M acetic acid (50 mL/h). Fractions containing pea vicilin subunits in the chromatographic profile (as well as in that of high-performance liquid chromatography (HPLC)) were detected by immunodetection with a rabbit polyclonal antibody against lentil vicilin Len c 1.01 and with a pool of sera from pea-allergic patients, after SDS-PAGE separation. These fractions were pooled, dialysed, freeze-dried and further separated by reverse-phase HPLC on a Nucleosil-C4 column (0.8×25 cm; particle size $5\ \mu\text{m}$; Scharlau Science, Barcelona, Spain), eluting with a three-step linear gradient of acetonitrile in 0.1% trifluoroacetic acid (30–40% in 40 min, 40–60% in 160 min and 60–85% in 15 min; 1 mL/min).

The protein content of the chromatographic fractions were determined by the bicinchoninic acid method [17].

Table 1. Clinical and demographic data of selected patient with pea allergy

Patient no.	Age (years)/sex	Symptoms	Total IgE (kU/L)	Pea-specific IgE (kU/L)	Sensitization to other legumes
1	12/F	URT	270	17.9	ln, cp, pn, sb, wb
2	25/M	URT	238	5.12	ln
3	9/F	URT	1158	6.36	ln, cp, pn
4	6/M	URT	1041	7.94	pn
5	13/M	URT	410	6.82	ln, cp, wb
6	21/M	URT	441	5.95	pn
7	22/M	URT	199	23.2	ln, cp, pn
8	7/M	URT	239	3.96	ln
9	6/M	URT, V	938	16.2	pn, wb
10	15/M	URT	464	17.5	ln, pn, cp
11	11/M	URT, OAS	1186	15.5	ln, wb, pn
12	7/F	URT, OAS	958	5.98	pn, wb
13	2/F	URT, OAS	310	5.65	ln, cp, pn, sb
14	3/M	URT	168	40.4	ln, cp, pn
15	8/M	URT	496	7.49	ln, cp, sb
16	10/M	URT, V	440	29.5	ln, cp, sb
17	13/M	URT	74	27.5	ln, cp, pn, wb
18	8/M	URT	966	11.8	ln, cp, pn, sb, wb

URT, urticaria-angioedema; V, vomiting; OAS, oral allergy syndrome; ln, lentil; cp, chickpea; pn, peanut; sb, soybean; wb, white bean.

The lentil allergen and vicilin Len c 1.01 was isolated as previously described [10].

Identification of pea convicilin, vicilin and its proteolytic subunits

Protein preparations enriched in pea vicilins were separated by SDS-PAGE according to Laemmli [18] on Bio-Rad Miniprotein II System gels (15% polyacrylamide) (Bio-Rad, Hercules, CA, USA). Proteins were then electrotransferred onto polyvinylidene difluoride (PVDF) membranes, as previously reported [19], and revealed by Coomassie blue staining. Appropriate individual bands (those binding specific IgE) were sliced and subjected to N-terminal amino acid sequencing using standard methods with an Applied Biosystems 477A gas-phase sequencer (Applied Biosystems, Foster City, CA, USA).

Immunodetection and immunoblot inhibition assays

Samples (20 µg of protein of pea extracts, 15 µg of protein of gel filtration fractions or 5 µg of protein of reverse-phase HPLC fractions) were fractionated by SDS-PAGE and then electrotransferred to PVDF membranes. After washing and blocking, membranes were treated with a serum pool (4 mL/gel) or individual sera (1 mL/sample) from patients with pea allergy, diluted 1:5 with PBS buffer (0.1 M Na phosphate, 0.15 M NaCl, pH 7.4, 0.1% (w/v) defatted milk) or with control sera (1:5 dilution). Membranes were then washed with PBS buffer (4 ×) and with tris-buffered saline (TBS) buffer (20 mM Tris, 0.15 M NaCl, pH 8.2, 0.1% (w/v) defatted milk; 3 ×), and then incubated with alkaline phosphatase-conjugated monoclonal anti-human IgE (clone GE-1, Sigma-Aldrich, St. Louis, MO, USA; 1:500 dilution in TBS buffer). After washing with TBS buffer (3 ×), reactive bands were revealed by adding 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium solution.

Alternatively, replica membranes were incubated with rabbit polyclonal antisera to lentil Len c 1.01 (an in house preparation obtained by standard methods; 1:5000 dilution in TBS buffer), washed with TBS buffer (4 ×), treated with alkaline phosphatase-conjugated anti-rabbit IgE (Sigma; 1:500 dilution in TBS buffer), washed again with TBS buffer (3 ×) and finally revealed with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium solution.

Immunoblot inhibition assays were carried out by the same method, except that 1 mL of sera were pre-incubated for 3 h at 25 °C with 10 µg of each inhibitor (purified Len c 1.01, the gel-filtration fraction enriched in pea vicilins or bovine serum albumin (BSA) as negative control).

Isolation of cDNAs encoding pea vicilin

Total RNA from mature pea seeds was obtained by the method of Chang et al. [20]. The reverse transcription was performed with 5 µg of total RNA using the primer 5'-TCGACTCTAGAGGATCCGAATTCAAGC(T)₁₅-3' and the first-strand cDNA Synthesis Kit (Amersham Biosciences). The cDNA obtained was amplified by PCR using the oligonucleotide 5'-primer 5'-CCTCGAATTCTCTAGATCCGATCAAGAGAACCCC-3', and for the reverse direction the 3'-primer 5'-AAGCTTTTACTCTCTTTGCAGAGG-3'.

Both oligonucleotides were designed from the nucleotide sequence of a cDNA encoding pea vicilin previously reported [21] (accession number X67429). The amplification was carried out with AmpliTaq Gold polymerase (Roche Molecular Biochemicals, Indianapolis, IN, USA) as recommended by the manufacturer. The PCR program was 10 min denaturation at 95 °C, followed by 35 cycles each of 30 s denaturation at 95 °C, 30 s annealing at 65 °C and 2 min extension at 72 °C and final extension for 7 min at 72 °C.

The PCR products were ligated into pGEM T-Easy vector (Promega, Madison, WI, USA). These constructs were used to transform *Escherichia coli* XL1-Blue cells, and the DNAs from several selected clones were sequenced using the ABI PRISM Dye Terminator Kit and ABI 3100 sequencer (Perkin-Elmer Biosystem, Foster City, CA, USA).

Results

The SDS-PAGE separation followed by protein staining of the crude and boiled pea PBS extracts revealed complex electrophoretic patterns, including components ranging from around 100 to 5 kDa (Fig. 1a). The main differences between both extracts were an increase of low-molecular-weight bands below 20 kDa and a decrease of components above 65 kDa in the extract from heat-treated seeds (Fig. 1a, lane 2), a result in line with those previously reported for lentil [9, 10]. The detection of vicilin and related proteins in the pea extracts was achieved by immunodetection with rabbit polyclonal antiserum developed against the lentil vicilin allergen Len c 1.01 [10]. Four major bands with apparent molecular sizes of 63, 44, 36 and 32 kDa and a minor one of 13 kDa were detected (Fig. 1b). Interestingly, electrophoretic heterogeneity was shown in the 44 kDa band. Three of the protein bands recognized by the rabbit anti-vicilin antibodies, namely those of 63, 44 and 32 kDa, were revealed to be as the most relevant IgE binding components by testing a pool of sera from

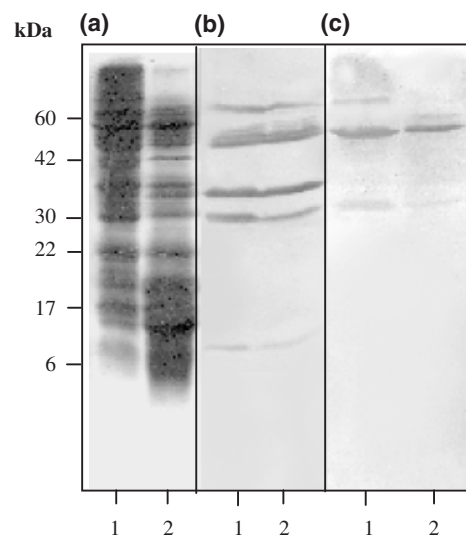


Fig. 1. Protein staining (a) and immunodetection with rabbit anti-vicilin serum (b) and with a serum pool from 18 patients with pea allergy (c) of crude (1) and boiled (2) extracts from pea seeds separated by SDS-PAGE.

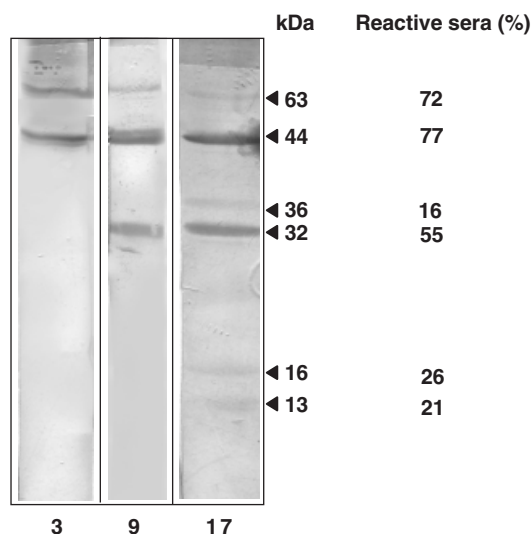


Fig. 2. IgE immunodetection patterns obtained with individual sera from patients 3, 9 and 17 (see Table 1). Apparent molecular weights of the reactive bands and percentages of individual sera recognizing each of them are summarized on the right-hand side of the figure.

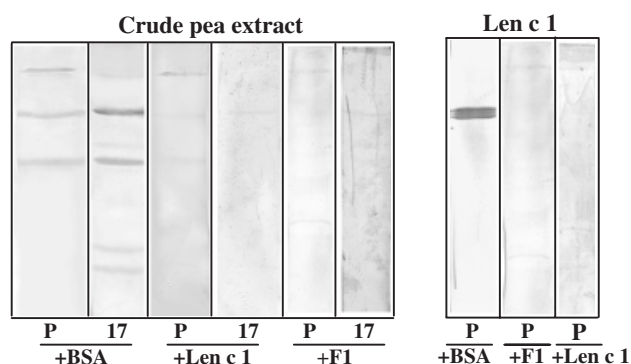


Fig. 3. Immunoblot inhibition assays with bovine serum albumin (BSA) (negative control), purified Len c 1.01 or a gel-filtration fraction enriched in pea vicilins (F1; see Fig. 4) as inhibitor. The crude pea extract or purified Len c 1 electrotransferred onto polyvinylidene difluoride membranes after SDS-PAGE separation was immunodetected with a serum pool from patients with pea allergy (P) or with serum from patient 17 (see Table 1), previously incubated with each inhibitor.

patients with pea allergy in specific IgE immunodetection assays (Fig. 1c). The same bands were recognized in the crude and heat-treated extracts by both the anti-Len c 1 serum and the pool of sera from allergic patients. Negative control sera did not react with any band when the two pea extracts were analysed.

A survey of the 18 individual sera forming the pool used in Fig. 1c revealed different patterns of IgE recognition. The patterns displayed by the serum corresponding to patients 3, 9 and 17 (Table 1) are shown as representative examples in Fig. 2. Comparison of the 18 binding patterns revealed six strongly IgE-reactive protein bands. As illustrated in the representative example in Fig. 2, three of these, the 63, 44 and 32 kDa bands previously located by using the serum pool, reacted with more than 50% of the individual sera, whereas the remaining three, corresponding to the 36, 16 and 13 kDa bands, were only detected by 16–26% of the sera tested.

The recognition of most pea IgE binding components by anti-Len c 1.01 antibodies (Figs 1b and 2) suggested

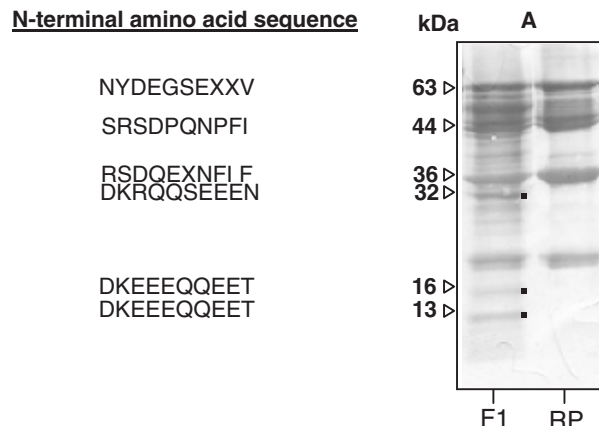


Fig. 4. N-terminal amino acid sequences of the IgE binding proteins from pea extracts belonging to the vicilin family. Gel-filtration (F1) or reverse-phase high-performance liquid chromatography (RP) fractions enriched in such proteins were separated by SDS-PAGE and electrotransferred onto polyvinylidene difluoride membranes. Bands sequenced from each fraction are marked with a point.

an immunological and structural relationship between the pea reactive proteins and the lentil allergen. Two different experimental approaches were undertaken to support such relationship. Firstly, immunoblot inhibition assays using purified Len c 1.01 as an inhibitor showed a strong (or even full) decrease of IgE binding to all pea reactive bands (Fig. 3), indicating the presence of common IgE epitopes with Len c 1.01. This immunological relationship was fully confirmed by the strong inhibition of IgE binding to purified Len c 1.01 using a protein preparation enriched in pea vicilins as an inhibitor (Fig. 3). Secondly, N-terminal amino acid sequencing of the putative pea allergens (Fig. 4) confirmed the homology of the sequences determined for the 44 and 36 kDa bands with the N-terminal sequence of the lentil allergen belonging to the vicilin family.

To further characterize the pea IgE binding proteins, as well as to obtain the complete amino acid sequence of pea vicilin, two cDNAs encoding members of this protein family were isolated by PCR amplification using non-degenerated oligonucleotide primers (see Materials and methods). The nucleotide sequences of the coding regions for both mature vicilin variants, named Pis s 1.0101 (accession no. AJ626897) and Pis s 1.0102 (accession no. AJ626898) according to the protocol of the International Committee of Allergen Nomenclature, are summarized in Fig. 5. Both cDNAs included a 1246 bp open-reading frame encoding for 415 amino acid proteins (Fig. 6), which showed 99% of sequence identity (changes Pis s 1.0101/Pis s 1.0102: His 56 Arg, Tyr 63 Cys and Ala 123 Thr). Theoretic molecular weights and isoelectric points of 47 278 Da and 5.42, and of 47 297 Da and 5.43 were calculated for Pis s 1.0101 and Pis s 1.0102, respectively. Consideration of both the alignments of the deduced Pis s 1.0101 amino acid sequence, the N-terminal amino acid sequences of the pea IgE binding proteins (Figs 6a and b), and the apparent molecular sizes of such proteins allowed to clarify the nature of the potential pea allergens. The 44 kDa band could be identified as mature pea vicilin, whereas other reactive bands were found to be proteolytic fragments derived from it. Considering the known characteristics of pea vicilin subunits [13, 22], the 36 kDa band probably comprised the

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Pis s 1.0101,2 tct aga tcc gat caa gag aac ccc ttt atc ttt aag tct aac cga ttt caa act ctt tat 60
Pis s 1.0101,2 gag aac gaa aac ggt cac att cgt ctt ctc caa aaa ttt gac aaa cgt tcc aaa ata ttt 120

Pis s 1.0101 gaa aat ctt caa aat tac cgt ctt tta gaa tat aag tcc aaa cct cac acc ctt ttt ctt 180
Pis s 1.0102 g 180

Pis s 1.0101 cca caa tac acc gat gcc gac ttc atc ctt gta gtc ctt agt ggg aaa gcc act ctc aca 240
Pis s 1.0102 g 240

Pis s 1.0101 gtg ttg aaa tct aac gat cga aac tcc ttc aat ctt gaa cgt ggt gat gcc atc aaa ctc 300
Pis s 1.0102 a 300

Pis s 1.0101,2 cct gct ggc act att gct tat ttg gct aac cga gat gac aac gag gat ctt aga gta tta 360

Pis s 1.0101 gat ctc gcc atc cca gta aac aaa ccc ggt caa ttg cag tct ttc tta tta tct gga act 420
Pis s 1.0102 a 420

Pis s 1.0101,2 caa aat caa cca tca tta tta tct gga ttc agc aag aat att cta gag gct gct ttc aat 480
Pis s 1.0101,2 acc aat tac gag gag ata gaa aag gtt ctt tta gaa caa cag gaa caa gag cca caa cac 540
Pis s 1.0101,2 aga aga agt ctt aag gat agg aga caa gag atc aac gaa gaa aat gta ata gtc aaa gta 600
Pis s 1.0101,2 tca agg gaa caa att gag gaa ttg agc aaa aat gca aag tcc agt tcc aaa aaa agt gta 660
Pis s 1.0101,2 tca tca gaa tct gga cca ttc aac ttg aga agt cgc aat cct atc tat tct aac aag ttt 720
Pis s 1.0101,2 ggc aaa ttc ttt gag atc acc cca gag aaa aat caa caa ctt caa gac ttg gac ata ttt 780
Pis s 1.0101,2 gtc aat tct gtg gat att aag gag gga tct tta ttg ttg cca aac tac aat tca aga gca 840
Pis s 1.0101,2 att gtg ata gta act gtt acc gaa gga aaa gga gat ttt gaa ctt gtg ggt caa aga aat 900
Pis s 1.0101,2 gag aac cag gga aaa gaa aat gac aag gaa gag gaa caa gaa gaa gag aca agc aaa caa 960
Pis s 1.0101,2 gtg caa ctg tat aga gct aag ttg tct cca ggt gat gtt ttt gtg att cca gca ggt cac 1020
Pis s 1.0101,2 ccc gtt gcc ata aat gcc tcc tca gat ctc aat ctg att gga ttt ggt atc aat gcc gag 1080
Pis s 1.0101,2 aac aac gag aga aac ttc ctt gca ggt gag gaa gac aat gtc ata agt caa gta gaa aga 1140
Pis s 1.0101,2 cca gtt aaa gag ctt gca ttt cct gga tct tct cat gag gtt gat agg ctc cta aag aat 1200
Pis s 1.0101,2 caa aaa caa tct tat ttt gca aat gct cag cct ctg caa aga gag taa 1248

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Fig. 5. Nucleotide sequences of the coding regions encoding the mature pea vicilin variants Pis s 1.0101 and Pis s 1.0102. Only sequence differences between both coding regions are shown.

$\alpha + \beta$ subunits (residues 1–307), the 32 kDa band comprised the $\beta + \gamma$ subunits (residues 185–415) and the 16 and 13 kDa bands comprised the γ subunit (residues 307–415; see Figs 6a and b).

Interestingly, a close structural relationship between Pis s 1 and Len c 1 was highlighted (Fig. 6a; 90% of amino acid sequence identity). Moreover, a significant but weaker similarity was found with other legume allergens of the vicilin family, such as peanut Ara h 1 (52% of sequence identity).

Finally, the 63 kDa reactive band was identified as pea convicilin (Fig. 6c), a family of 68–64 kDa seed storage proteins immunologically related to vicilin, with which it shows a high degree of sequence identity [13, 23]. Pea convicilin has been named Pis s 2, according to the proposal of the International Committee of Allergen Nomenclature.

Discussion

An increasing body of clinical evidence points to a substantial allergenic cross-reactivity among lentil, chickpea and pea in the Mediterranean area, where these legumes are widely consumed [3, 4, 8]. This fact contrasts with the low clinical significance of legume cross-reactions (mainly between peanut and soybean) reported in the United States [7]. To further support the lentil/chickpea/pea cross-reactivity mentioned above, at least two different experimental lines should be pursued. The main allergens of the three legumes should be identified, isolated and characterized, and *in vitro* assays should be performed to evaluate their potential cross-reactions. On the other hand, large groups of patients allergic to more than one of these legumes (confirmed by DBPCFC or open challenges) should be tested *in vivo* using the purified allergens. With respect to the first approach, only the major allergens of lentil have been identified and characterized in the Mediterranean population [9, 10]. No similar data were available for chickpea and pea.

The major potential allergens from pea have been identified here by using sera from a group of patients with pea

allergy, and most of them (14 out of 18) also sensitized to lentil (Table 1). All the IgE binding proteins detected were closely related, and belonged to the 7S globulin family of seed storage proteins [13]. We have identified an initial set of allergens, jointly designated as Pis s 1, which includes mature vicilin chains of around 47 kDa, and several derived proteolytic fragments from 36 to 13 kDa. Because pea vicilins are encoded by a multigene family, is not surprising that several genetic variants are expressed in pea seeds of the same cultivar [13]. This fact could explain the electrophoretic heterogeneity found for the 44 kDa reactive band, as well as the minor differences between the amino acid sequences deduced from the cDNAs encoding vicilin variants, and between those and the N-terminal sequence determined for the 44 kDa protein band (Figs 4–6). Alignment of the complete amino acid sequences of pea vicilins and the N-terminal sequences obtained for the 36, 32, 16 and 13 kDa IgE binding proteins has also facilitated to identification of the reactive subunits derived from pea vicilin (Fig. 6). The N-terminal end of these sequences matched the proteolytic cleavage sites previously reported for pea vicilins [13, 22], thus determining their origin and suggesting a mode of processing. The IgE recognition patterns obtained by using individual sera from pea allergic patients (Fig. 2) point to mature vicilin and its 32 kDa proteolytic fragment as major pea allergens, whereas the 36, 16 and 13 kDa subunits would be minor ones.

Pis s 1 and Len c 1 show a high degree of sequence identity (90%), and also display a very similar pattern of post-translational processing [10, 13], thus indicating a closed structural relationship. A strong cross-reactivity between both allergens has been demonstrated by immunoblot inhibition assays (Fig. 3). These data suggest that allergenic vicilins can represent the molecular basis of the clinical co-sensitization to pea and lentil found in the Spanish population [4, 8]. Moreover, the immunological relationship of Pis s 1 and vicilins with other seeds, such as peanut Ara h 1 [14], could potentially extend the cross-reactions to different legumes depending on the feeding habits of the population studied.

(a)

44 kDa **SRSDPQNPF**I
 36 kDa **RSDQE** XNFIF
 Pis s 1.0101 **SRSDQENPF**IFKSNRFQTLYENENGHIRLLQKFDKRSKIFENLQNYRLLLEYKSKPHTLFL 60
 Len c 1.0102 I

Pis s 1.0101 PQYTDADFILVVLSGKATLTVLKSNDNRNSFNLERGDAIKLPAGTIAYLANRDDNEDLRVL 120
 Len c 1.0102 V N T

Pis s 1.0101 DLAI PVNKPQGLQSFLLSGTQNPQLLSGFSKNILEAAFNTNYEEIEKVLEQQEQEPQH 180
 Len c 1.0102 N E F N S D D

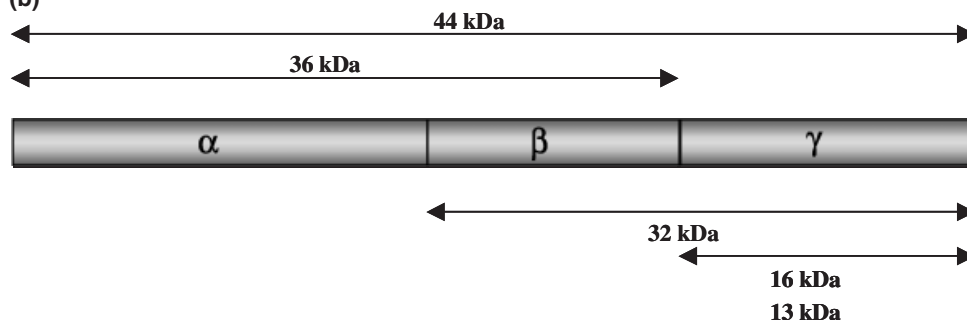
32 kDa **DKRQQSEEN**
 Pis s 1.0101 RRS LK**DRRQE**INEENVIVKVSREQIEELSKNAKSSSKSVSSSGPFLNRSRNPISYK 240
 Len c 1.0102 R K K E

Pis s 1.0101 GKFFEITPEKNQQLQDLDIFVNSVDIKEGSLLLPNYSRAIVITVTTEGKGDFELVGQRN 300
 Len c 1.0102 P E N Y

16 kDa/13 kDa **DKEEEQQEET**
 Pis s 1.0101 ENQ GKEND**DKEEEQ**EEETSKQVQLYRAKLSPGDV FV I PAGHPVA INASSDLNLIGFGINAE 360E
 Len c 1.0102 RE D T R V K

Pis s 1.0101 NNERNFLAGEEDNVISQVERPVKELAFPGSSHEVDRL LKNQKQSYFANAQPLQRE 415
 Len c 1.0102 Q IQ R T H I

(b)



(c)

Convicilin **NYDEGSE**TRVPG
 63 kDa **NYDEGSE**XXV--

A second type of pea allergen, named Pis s 2, which corresponds to the 63 kDa reactive band, has been identified as convicilin. Previous data indicated its closed structural similarity with the vicilin family [13, 23]. The recognition of Pis s 2 by anti-Len c 1 rabbit antibodies, and the inhibition of its IgE binding by the lentil allergen confirmed such a relationship. Pis s 2 seems to be a major pea allergen, based on the high percentage (72%) of individual sera that recognized it. Proteins from the convicilin family, such as the α subunit of β -conglycinin, are also potential allergens from soybean [24].

To further support the role of the vicilin family in food allergy and legume cross-reactions in the Mediterranean population, the chickpea homologue to Pis s 1 and Len c 1 should be isolated, and the purified allergens should be tested *in vivo* (SPTs) in patients with confirmed allergy to lentil, pea and/or chickpea.

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Fig. 6. (a) Alignment of the amino acid sequences of Pis s 1.0101, Len c 1.0102 (accession no. AJ551425) and the N-terminal sequences of the 44, 36, 32 and 13 kDa pea IgE binding proteins. Sequences matching between the protein and cDNA are in bold. Only sequence differences between Pis s 1.0101 and Len c 1.0102 are shown. (b) Schematic representation of Pis s 1.01, its α , β and γ subunits, and its correspondence with the 44, 36, 32, 16 and 13 kDa putative allergens. (c) Alignment of the N-terminal sequences of the 63 kDa IgE binding protein and pea convicilin (accession no. P13915).

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