

Identification and Characterization of Che a 1 Allergen from *Chenopodium album* Pollen

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Key Words

Chenopodium album · Chenopod pollinosis · Allergen · Che a 1 · Amino acid sequence · Ole e 1-like protein

Abstract

Background: Pollinosis to *Chenopodium album* has been reported, but no data are available on its allergenic proteins. **Methods:** An allergen from *C. album* pollen has been isolated by means of gel permeation and reverse-phase high-performance liquid chromatography. Molecular characterization was achieved by concanavalin A reaction, mass spectrometry, Edman degradation and cDNA sequence. Antigenic analyses were performed by immunoblotting, ELISA, and ELISA inhibition, using sera from allergic patients, two Ole e 1-specific monoclonal antibodies and an Ole e 1-specific polyclonal antiserum. **Results:** The isolated allergen, Che a 1, is a glycoprotein of molecular mass 17.088 kD and 143 amino acid residues, whose sequence exhibits 27–45% identity with known members of the Ole e 1-like protein family. 77% of sera from patients allergic to chenopod pollen were reactive to Che a 1. No correlation was found between the IgE reactivities to Che a 1 and Ole e 1, the major allergens from olive pollen, and both allergens display low, although detectable, IgE and IgG cross-reactivities. **Conclusions:** Che a 1, a relevant allergen from chenopod

pollen, is structurally related to the Ole e 1-like protein family, but exhibits significant differences on its polypeptide sequence that could explain its different antigenic behavior and limited cross-reactivity.

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Introduction

Chenopodiaceae are widespread weeds of temperate areas, which include allergenic members. Chenopods are predominantly spontaneous perennial plants growing on salt-enriched soils, but some species have also been used as hedges and ornamental plants, producing high local concentrations of pollen and being able to induce pollinosis [1–3]. In western USA and temperate countries of Europe, there are areas of significant pollen counts derived from Chenopodiaceae, although their allergenic effects can be masked by the presence of more prevalent species such as Gramineae or Oleaceae. Sensitization to pollen of *Beta vulgaris*, *Chenopodium album*, and *Salsola kali* has been described, but very little is known about their allergenic patterns [1, 4–9].

One of the most recognized allergenic members of the Chenopodiaceae family is *Chenopodium album*, which seems to have emerged in the last years mainly in desertic areas due to its use in greening programs. Areas of high

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incidence have been reported in the south of Spain, western USA, and Saudi Arabia [3, 5, 10–14], the pollination season lasting from June to October. In desert environments, such as Kuwait, Chenopodiaceae pollens are the main sensitizing agents among patients with allergic rhinitis or asthma with prevalence values higher than those for dusts or molds [10–13]. However, scarce data are available on *C. album* pollen allergens, since low reactivity was detected in immunoblotting using both individual sera from sensitive patients [9] and pools of patient sera [1, 8].

Polysensitization is usually observed in patients affected by chenopod pollinosis. Although the presence in the environment of more relevant allergenic pollens can explain this property, the existence of common allergens shared by chenopod and other different pollen sources cannot be excluded. In fact, several authors have demonstrated a degree of cross-reactivity among pollen extracts from Chenopodiaceae members and those of the taxonomically related Amaranthaceae family [3, 8, 9]. Such studies concluded that Chenopodiaceae pollens contain common IgE-binding antigens, although there are also specific allergens. It is well known that cross-reactivity can also be displayed by allergens from unrelated biological sources, and thus the pollen of *C. album* could share common allergens with pollens of not closely related species such as Gramineae, Betulaceae or Oleaceae.

On the basis of observations from our group about the existence of a putative correlation between the sensitivity to *C. album* and Ole e 1, the major allergen from olive pollen, we analyzed the existence of an Ole e 1-like allergen in this species.

Materials and Methods

Patient Sera and Ole e 1-Specific Antibodies

The sera used in this study were obtained from 71 untreated patients exhibiting pollinosis to *Olea europaea* and *C. album* pollen with seasonal rhinitis and/or bronchial asthma, positive skin test responses to *O. europaea* and *C. album* pollen extracts. Sera were class 3–6 and 1–4 (CAP system from Kabi Pharmacia, Uppsala, Sweden), respectively, for olive and chenopod pollen extracts. A pool of sera from nonallergic individuals was used as negative control.

Polyclonal antiserum against Ole e 1 was prepared by immunizing a New Zealand White rabbit over a 6-week period by injection of the natural allergen (100 µg) in complete Freund's adjuvant [15]. Ole e 1-specific monoclonal antibodies (OL3 and OL9) were prepared as described [16].

Purification of Che a 1 and Ole e 1

Pollens from *C. album* and *O. europaea* were obtained from ALK-Abelló (Madrid, Spain). Pollens (5% w/v) were extracted with

50 mM ammonium bicarbonate, pH 8.0, containing 1 mM phenylmethylsulfonyl fluoride, during 1 h at room temperature. Ole e 1 was isolated according to Villalba et al. [17]. Che a 1 was isolated by the same procedure as for Ole e 1 with minor modifications. Briefly, the supernatant obtained by saline extraction of proteins from the pollen was lyophilized and chromatographed into a Sephadex G-75 size exclusion column, which was eluted in 0.2 M ammonium bicarbonate. Fractions were analyzed by SDS-PAGE and immunoblotting against Ole e 1-specific antibodies, and those with positive reaction were pooled, lyophilized and separated by reverse-phase high-performance liquid chromatography (RP-HPLC) in a µ-Bondapak C-18 column, eluted with an acetonitrile gradient in 0.1% (v/v) trifluoroacetic acid [17]. Fractions were monitored at 214 nm wavelength, and the presence of Ole e 1-like protein was analyzed by immunoblotting after SDS-PAGE. Protein concentration was determined according to Lowry et al. [18].

Analytical Procedures

The amino acid composition of the purified protein (1–2 nmol) was determined by quantitative amino acid analysis after hydrolysis with 5.7 N HCl at 105 °C for 24 h, in tubes sealed under vacuum [17]. N-terminal Edman degradation of the purified protein was performed using an Applied Biosystems 491 sequencer. Mass spectrometry analysis was carried out as described [15], in a Bruker Reflex II MALDI-TOF mass spectrometer (Bruker-Franzen Analytik, Bremen, Germany). The equipment was externally calibrated employing single-, double- and triple-charged signals from either cytochrome c (12,360 D) or bovine serum albumin (66,430 D).

SDS-PAGE was performed according to Laemmli [19] in 15% (w/v) polyacrylamide gels under nonreducing conditions. Proteins were visualized by Coomassie Brilliant Blue R-250 staining or alternatively transferred to membranes. The molecular mass of protein bands was estimated by comparison with protein markers (MW-SDS-70L) provided by Sigma.

Carbohydrate Detection

Carbohydrate detection of purified protein was performed, after electrophoresis and transfer of the samples to nitrocellulose membranes, by reaction with biotinylated concanavalin A (Con A) lectin, as described [20]. The staining was developed by horseradish peroxidase reaction with 0.05% (v/v) 3,3'-diaminobenzidine/HCl, 0.03% (v/v) of 30% (v/v) H₂O₂ in Tris/NaCl reaction buffer.

cDNA Cloning and Nucleotide Sequencing of Che a 1-Specific cDNA

Total RNA from chenopod pollen was obtained as described [21]. First-strand cDNA was synthesized from 2.5 µg of RNA using the SMART™ RACE cDNA amplification kit (Clontech). A kit adaptor primer [15] and a degenerate oligonucleotide which was designed from an amino acid segment (CRIQFMT) of the sequence obtained by Edman degradation (CH1, 5' TGYCGNGTNCARTTYATGAC 3') were used to amplify a partial sequence of the specific cDNA. The nucleotide sequencing of this fragment allowed to obtain the C-terminal end sequence of the protein. A primer designed from this sequence (CH2, 5' cgctccgcgTAAATTAGCTTTAACATCATAAAGATC 3') and the same kit adaptor primer as mentioned above were used to obtain the 5' end of the encoding sequence. Finally, the use of the primers corresponding to both ends of the protein, CH2 and CH3 (CH3: 5'cgctctcgagaaaagaGCCGAGAACCATTTCAAAGTC 3') rendered the full-length encoding sequence. The obtained cDNA frag-

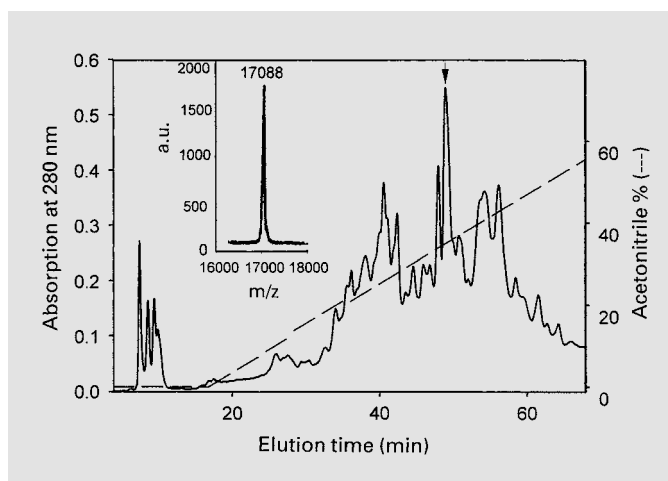


Fig. 1. Elution profile of the last chromatographic step to isolate the chenopod allergen on a μ -Bondapak C-18 (RP-HPLC) column using an acetonitrile gradient. The arrow indicates the elution of Che a 1. **Inset:** mass spectrometry analysis of Che a 1 in arbitrary units (a.u.).

ments were purified and cloned into the pCR 2.1 vector included in the TOPO TA cloning kit (Invitrogen, Groningen, The Netherlands). The nucleotide sequences were obtained according to Villalba et al. [21].

Immunoblotting Analysis

Proteins separated by SDS-PAGE were transferred to nitrocellulose membranes (Bio-Rad, Richmond, USA) in 48 mM Tris-HCl, pH 9.0, containing 39 mM glycine, 0.0375% (w/v) SDS and 20% (v/v) methanol. Immunodetection was carried out as described [22]. The nitrocellulose strips, after treatment with PBS-0.1% (v/v) Tween 20 for 15 min and blocking with 3% (w/v) skimmed milk in PBS-0.1% (v/v) Tween 20 for 1 h, were probed with individual sera from olive-allergic patients or with a pool of these sera (diluted 10-fold), for 2 h, at room temperature. Strips were then washed with 0.1% (v/v) Tween 20 in PBS and incubated for 1 h with a mouse antihuman IgE diluted 5,000-fold in PBS containing 0.05% (v/v) Tween 20 and 1.5% (w/v) skimmed milk. After washing, blots were incubated for 1 h with horseradish peroxidase-labeled goat anti-mouse IgG (Pierce Chemical Co., Rockford, USA), diluted 1,500-fold. The peroxidase reaction was developed by means of the excited-chemiluminescence Western-blotting reagent and ECL-hyperfilm (Amersham, UK).

Alternatively, the membranes were reacted with Ole e 1-specific monoclonal antibodies (diluted 5,000-fold) or with a rabbit polyclonal antiserum (diluted 3,000-fold) raised against Ole e 1, as described [15].

Quantification of IgE Antibodies

IgE-binding measurements of allergic sera were performed by titration of solid phase-fixed antigens, as described [23]. Briefly, ELISA plates were coated with 100 μ l/well of antigen (1 μ g/ml) in PBS. After washing and saturation with blocking buffer, the plates

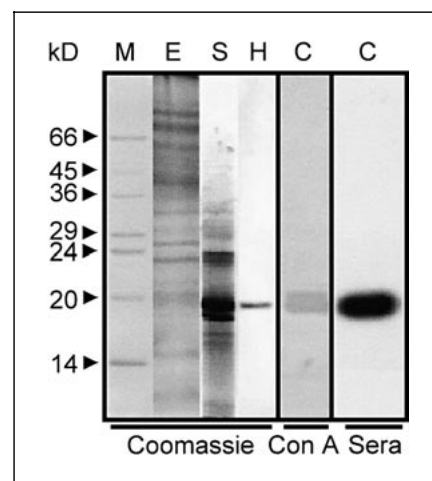


Fig. 2. SDS-PAGE analysis of Che a 1. Staining with Coomassie blue of: crude extract of *C. album* (E), sample eluted from the size exclusion column (S), Che a 1 eluted from RP-HPLC (H) and the sample of lane H stained for sugars with Con A lectin, or immunostained with a pool of allergic sera for assay the IgE-binding activity (C). M = Molecular mass markers.

were incubated with the human sera (diluted 10-fold) followed by mouse antihuman IgE and horseradish peroxidase-labeled goat anti-mouse IgG. Finally, the peroxidase reaction was developed using freshly prepared enzyme substrate and measuring the optical density (OD) at 492 nm. The mean value of three determinations for a pool of sera from nonallergic subjects was regarded as the negativity cut-off. Pearson's linear regression coefficients (r) were determined.

ELISA Inhibition

ELISA inhibition assays were performed as described [15]. Briefly, after coating with 100 μ l of Che a 1 (1 μ g/ml), the plates were incubated with a pool of four sera (diluted 10-fold) from patients allergic to chenopod and olive pollen, which possessed similar specific IgE values for both, Che a 1 and Ole e 1. The pool of sera was preincubated with different concentrations of nOle e 1, rOle e 1 and Che a 1 as inhibitors. The response was developed by incubation with mouse antihuman IgE (diluted 5,000-fold) kindly donated by Dr. Lombardero (ALK-Abelló) and horseradish peroxidase-labeled goat antimouse IgG (Pierce Chemical Co.) diluted 2,500-fold.

Results

Isolation of Che a 1

An Ole e 1-specific polyclonal antiserum was used in immunoblotting as a tool in order to isolate the Ole e 1-like protein from chenopod pollen extract. The procedure to purify the protein was similar to that previously used for the olive pollen allergen. After two chromatographic steps, gel permeation and RP-HPLC (fig. 1), a highly puri-

1	ATGGCGAAGTGTCAGCTGTTTTCTTTTGGTTGGCGCTCTCTGCGTCTGTCCTTGGCCGGTGTAGCCAATGCC	
	M A K C Q A V F L L V G A L C V L S L A G V A N A	25
76	<u>GCCGAGAACCATTTC</u> <u>AAAGTCCAGGGCATGGTGTACTGTGACACTT</u> <u>GCCGTATCCAATTTATGACCCGCATTAGT</u>	
	A E N H F K V Q G M V Y C D T C R I Q F M T R I S	50
151	ACAATAATGGAAGGGCAACTGTGAAATTGGAATGCAGAAACATTACTGCAGGAAGTCAGACCTTCAAAGCTGAA	
	T I M E G A T V K L E C R N I T A G T Q T F K A E	75
226	GCTGTAAGTATAAGGTAGGACAGTACAGCATCCCTGTTAATGGTGATTTCGAAGACGATATCTGTGAAATCGAG	
	A V T D K V G Q Y S I P V N G D F E D D I C E I E	100
301	TTGGTTAAGAGCCCCAACAGCGAATGCTCTGAGGTTTCACATGATGTTTATGCCAAGCAATCTGCTAAGGTTAGC	
	L V K S P N S E C S E V S H D V Y A K Q S A K V S	125
376	CTAACATCCAACAATGGTGAAGCTTCAGACATTGCGAGCGCCAATGCTCTCGGATTTCATGAGGAAGGAGCCCTT	
	L T S N N G E A S D I R S A N A L G F M R K E P L	150
451	AAAGAGTGCCTGAGGTTCTCAAGGAGTTGGATCTTTATGATGTTAAAGCTAATTAA	
	K E C P E V L K E L D L Y D V K A N *	168

Fig. 3. Nucleotide sequence of the cDNA fragment encoding Che a 1 and deduced amino acid sequence. The oligonucleotide segments used as primers are underlined. The signal peptide is shown by a dotted line. The consensus N-glycosylation site is boxed. The stop codon is indicated by an asterisk.

fied protein was obtained. SDS-PAGE analyses of different steps of the purification procedure are shown in figure 2. The procedure rendered 0.2 mg of pure protein per gram of dried pollen. The analysis by immunoblotting of the binding of the purified protein to IgE antibodies from a pool of sera from patients allergic to chenopod pollen was found to be positive (fig. 2) and, therefore, the antigen was called Che a 1, according to the Allergy Nomenclature Subcommittee of the International Union of the Immunological Societies [24].

Molecular Characterization

Che a 1 displayed an apparent molecular mass of 18.8 kD in SDS-PAGE (fig. 2), and a value of 17.088 kD by mass spectrometry analysis (fig. 1, inset). The positive reaction of Che a 1 with Con A lectin indicated its glycosylated character (fig. 2). The amino acid composition of Che a 1 was determined after acidic hydrolysis and automatic analysis (data not shown). Edman degradation of Che a 1 rendered 22 amino acid residues of the N-terminal sequence of the protein: AENHFQVQGMVYCDTCRVQFMT.

cDNA Cloning and Nucleotide Sequencing

Total RNA was obtained from chenopod pollen and a single-stranded cDNA was synthesized. This cDNA was used as template to amplify by PCR a DNA fragment encoding Che a 1 using the oligonucleotides underlined in the nucleotide sequence of Che a 1 in figure 3. The

deduced amino acid sequence corresponded to an open reading frame of 168 residues in length. It contains a signal peptide of 25 amino acids as it is derived from the data obtained by Edman degradation of the isolated allergen. A potential N-glycosylation site (NXT/S) appears at Asn-64 of the preprotein (Asn-39 of the mature protein). The comparison of the sequence of Che a 1 with nucleotide sequences from databases allowed to detect homology to the Ole e 1-like protein family (fig. 4). The identity of the polypeptide sequence of Che a 1 with members of this family of proteins ranges between 27% for the protein of plantain and 45% for that of birch.

Significance of Che a 1-Specific IgEs in the Response to the Whole Chenopod Extract

We performed ELISA experiments with 71 serum samples of patients who showed allergic symptoms both to chenopod and olive tree pollen as well as positive skin prick tests to their extracts, in order to measure the IgE reactivity to chenopod pollen extract and purified Che a 1 (fig. 5a). 87% of these sera exhibited a positive IgE reactivity to the whole extract and 77% to the isolated allergen. The OD values were low both for the pollen extract and for purified Che a 1 (Pearson's coefficient $r = 0.017$). This would agree with the low RAST values (classes 1–3) obtained for these sera with chenopod pollen extract. These results suggest that Che a 1 is a relevant allergenic component of the chenopod pollen.

Che a 1	-----AE-NHFVKVQGMVYCDTCRIQ-FMTRISTIMEGATVK	34
Ole e 1	-----EDIPQPPVSQFHIQGVYCDTCRAG-FITELSEFIPGASLR	40
Lig v 1	-----EDVPQPPVSQFHIQGVYCDTCRAR-FITELSEFIPGAVR	40
Lol p 11	-----DKGPGFVVTGRVYCDPCRAG-FETNVSHNVEGATVA	35
Pla l 1	-----TQTSHF--AKFHVEGEVYCNVCHSRNLINELSERMAGAQQV	39
Syr v 1	-----EDVPQPPVPQFHIQGVYCDTCRAR-FITELSEFIPGASIR	40
Atal	-----MSKAVLLVALCFLPALAIAARPKNPFVXGRVYCDTCXVX-FETPASTYISGAVVR	56
Bpen	-----MAKSIIIQAPALCFLSLGFAISE-SRFEVEGKVYCDNCRQ-FVTKLSTYMKAKVS	56
Lesc	-----MAKAIVLLS-ALCILALANFAHCRPEVDFVEGKVYCDTCRVQ-FETKLSNLEGGATVK	56
Osat	MASL-----RTIPVIFGILFYVLASTATADAPDYVVGGRVYCDTCRAE-FETNVTEYIKGAKVR	59
Snig	-----MARLILLVALSFLP-LLTSARLIGNPFLKGSVYCDTCRAG-FETDATKYLHGAVR	55
Zmay	MASVPAPATTTAAVILCLCVVLSCAAADDPNLPDYVIQGRVYCDTCRAG-FVTNVTEYIKGAKVR	64

Che a 1	LECRNITAGTQTFKAEAVTDKVGQMSIPVNGDFEDDICEIELVKSPNSECEVSHDVIYAKQSAK-	98
Ole e 1	LQCKDKENGDTVTFEVGYTRAEGLYSMLVERDHKNEFCEITLSSSRKDCNEIP--TEGWAKPS-	102
Lig v 1	LQCKDGENGKITFTEVGTYRAEGLYSMLIERDHKNEFCEITLSSSRKDCNEIP--IEGWVKPS-	102
Lol p 11	VD CRPFDGGESKLKAEATDKDKGNYKIEIQDQHQEIECEVVLAKSPDKSCSEIE---EFRDRAR-	96
Pla l 1	LQCKD--DSKKVIYSIGGETDQDGVYRLPVGYHED--CEIKLVKSSRPDCSEIPKLAKGTITQTSK	101
Syr v 1	LQCKDGENGKITFTEIGYTRAEGLYSMLVEGDHKNFCEITLSSSRKDCNEIP--VEGWVKPS-	102
Atal	LECKDRRTMELTYSHEARTDSTGSKILVNEDHDEQFCDAMLVRSQRLCSNVS---PGHDRAR-	117
Bpen	LECRNREGGTLIYSSDSETDKSGTYRIPVDGDHEEIECEIAKKSSDPDCSEVSKDPFLKKSAR-	120
Lesc	LQCRNISTEAETFSVEGVTDKDKGKYKLTVNGDHENDICEVTVKSPREDCKESVSG---YEKAR-	117
Osat	LECKHFGTDKVERAIDGVTDGTGYKIELKDSHEEDICEVVLVHSPLANCSEIE---AERDRAR-	120
Snig	IECKDRDTRLRTYSDEAVTDSKGYSVLVSDDHGDQMCDAILVSSPDPECAVPD---KGRDRAC-	116
Zmay	LECKHFGTGKLERAIDGVTDATGTYTELEKDSHEEDICQVVLVASPRKDCDEVQ---ALRDRAG-	125

Che a 1	VSLTSNNGEASD-IRSANALGFMKEPLKECPEVLKELDLY--DVKAN	143	
Ole e 1	LKFKLN-TVNGT-TRTVNPLGFFKKEALPKCAQVYNKLGMYPPNM---	145	30
Lig v 1	LKFKLN-TVNGT-TRTINPLGFFKKEALPKCPQVFNKLGMYPPNM---	145	31
Lol p 11	VPLTSNXGIKQQGIRYANPIAFFRKEPLKECGGILQAY-----	134	42
Pla l 1	VDLSKNTTITEK-TRHVKPLSFRAKTDAPGC-----	131	27
Syr v 1	LKFKLN-TVNGT-TRTINPIGFFKKEALPKCTQVYNKLGMYPPNM---	145	31
Atal	VTLTRFNGIASD-DRFANNMGFLRDAAMPGCADIMKLYQETE-----	158	33
Bpen	ISLTKNNGISTP-VRLANPLGFMKKKPLPECAKALRELGMNPDDVIQ-	166	45
Lesc	IECSDNVGIHNA-VRFANPLFFMKAESVQCKEALDELGLFPLEF---	161	38
Osat	VLLTRNVGICDN-LRLANPLGYLKDYHCP-SAALLKQFDLADDDNE--	164	30
Snig	VILTRNSGMTSD-ERFANAMGFLKNEPMAGCTQILQKYQLNEDD---	159	33
Zmay	VLLTRNVGISDS-LRPANPLGYFKDVPLPVCAALLKQLSDDDDDQ---	170	30

Fig. 4. Comparison of amino acid sequences of Che a 1 with members of the Ole e 1-like protein family. The mature proteins Che a 1 from *Chenopod album*, Ole e 1 from olive tree, Lig v 1 from privet, Lol p 11 from ryegrass, Pla l 1 from plantain and Syr v 1 from lilac have been reported as allergens. Their GenBank accession numbers are AY049012, X76395, CAA54819, A54002, CAC41633 and S43244, respectively. The remaining amino acid sequences were deduced from the corresponding encoding genes: Atal from *Arabi-*

dopsis thaliana, Bpen from *Betula pendula*, Lesc from *Lycopersicon esculentum*, Osat from *Oriza sativa*, Snig from *Sambucus nigra*, and Zmay from *Zea mays* (GenBank accession numbers AJ133639, CAA74365, P13447, S31710, AAF16869, P33050, respectively). Gaps (-) were introduced for optimizing the alignment. Residues conserved in all of the proteins are shaded in black. Residues conserved at least in ten chains are shaded in gray. Percentage of identity to Che a 1 is also indicated.

Analysis of Cross-Reactivity between Che a 1 and Ole e 1

The same 71 sera used above were analyzed to measure the IgE levels against Ole e 1 (fig. 5b). 57 sera (80%) were judged reactive to Ole e 1 and higher values of OD were obtained for Ole e 1 than for Che a 1, which indicates a

lower significance for Che a 1 in the titers of IgE of these sera. Moreover, no correlation was observed between the level of response of each serum to both allergens ($r = 0.085$), which suggests the absence of IgE-binding equivalence between them.

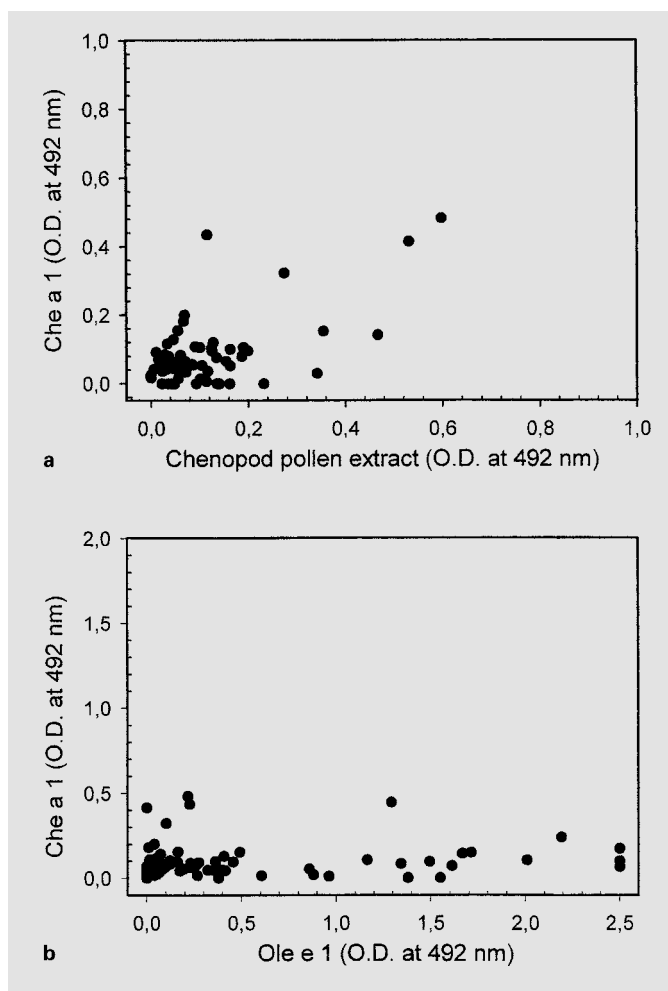


Fig. 5. Quantification of the levels of specific IgE by ELISA experiments. OD values at 492 nm of the IgE binding of the sera to Che a 1 versus those of the whole extract of chenopod (**a**) and those obtained for Che a 1 versus to those of Ole e 1 (**b**). Serum samples of 71 allergic patients were used. OD values <0.03 were considered as a negative response. Each value is the mean of two determinations.

IgE cross-reactivity between Che a 1 and Ole e 1 was analyzed by ELISA inhibition coating the wells with Che a 1. Both nOle e 1 and rOle e 1 were used as inhibitors for the preincubation of a pool of sera prepared with four sera which exhibited similar IgE titers for both allergens (fig. 6). The natural form of the olive allergen was able to inhibit 42% of the IgE binding to Che a 1 at 50 µg/ml of protein, and the recombinant form only reached 14% of inhibition at its highest concentration. Inhibition with Che a 1 was also performed as a positive control.

In order to compare the IgG-binding behavior of Che a 1 and Ole e 1, these proteins were analyzed by immuno-

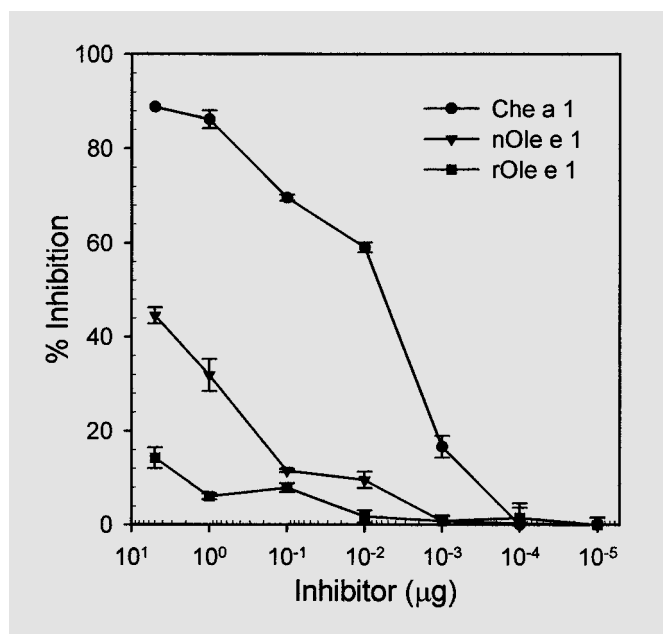


Fig. 6. ELISA inhibition of the IgE binding to Che a 1 fixed to solid phase. A pool of sera from patients allergic to both chenopod and Ole e 1 was preincubated with different amounts of nOle e 1 or rOle e 1 or Che a 1 as a positive control. Samples were prepared in duplicate; vertical bars indicate standard deviations.

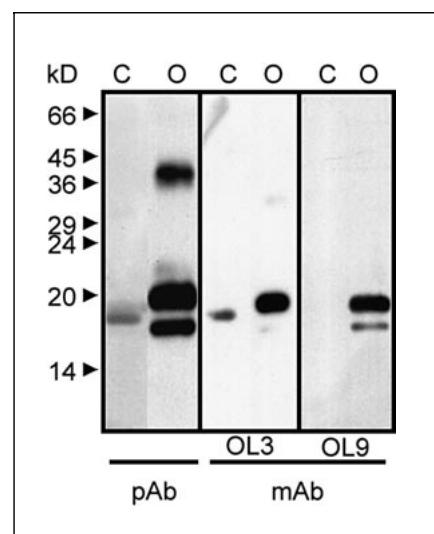


Fig. 7. Analysis of the binding of Ole e 1-specific antibodies to Che a 1 (1 µg, lanes C) and to Ole e 1 (1 µg, lanes O). Rabbit polyclonal antiserum (pAb), OL3 and OL9 monoclonal antibodies (mAb) were used for the immunostaining of the membranes. Ole e 1 presents up to three components: two glycosylated forms (monomer of 20 kD and dimer of 40 kD), and one nonglycosylated form (18.5 kD).

blotting after SDS-PAGE using one Ole e 1-specific polyclonal antiserum and two Ole e 1-specific monoclonal antibodies (OL3 and OL9). Figure 7 shows a positive response exclusively against Che a 1 with OL3, but remarkably lower than for Ole e 1, indicating a limited antigenic relationship between these two homologous proteins.

Discussion

Chenopod species have been recognized for many years as a cause of hay fever in warm countries [4–7]. However, little attention has been paid to the pollinosis they induce, probably because they are frequently associated with other allergenic sources of higher clinical relevance. Preliminary observations from our group suggested that the sensitivity to Ole e 1 in patients allergic to olive pollen could be correlated with sensitivity to *C. album* pollen. Moreover, the allergenic pattern of chenopod pollen extract showed a highly frequent band close to 20 kD when it was probed with individual sera from patients allergic to both olive and chenopod pollen. Therefore, we used an Ole e 1-specific polyclonal rabbit antiserum to isolate the Ole e 1-like protein from chenopod pollen extract. A glycoprotein with an apparent molecular mass of 18.8 kD was obtained. It was found to be able to bind IgE from sera of allergic patients and thus it was called Che a 1.

Early studies on chenopod pollen allergens had indicated that the allergen profile varies considerably depending on the method of analysis used, but always a very low number of allergens was detected [8, 9]. IgE-reactive bands of 55, 35 and 14 kD were found in *C. album* pollen extracts by Lombardero et al. [8] using SDS-PAGE and isoelectrofocussing. By crossed immunoelectrophoresis, Wurtzun et al. [9] identified 9 immunoprecipitated antigens, one of which could be classified as a major allergen, and only bands of 55, 25 and 15 kD were detected by fused-rocket immunoelectrophoresis. By using immunoblotting and staining with 20 sera, the same authors visualized only six bands in this extract (10.5, 14.5, 15, 40, 60.5, 68 kD), all of them with a frequency lower than 15%. The protein studied here, Che a 1, would represent an allergen not previously reported, although the high tendency of Ole e 1-like proteins to aggregate [20, 22, 25, 26] could account for bands previously detected around 35–40 and 55 kD [8, 9]. These data, along with our results, suggest the existence of a low measurable IgE reactivity for chenopod allergens, which could agree with the low

levels of chenopod-specific IgE present in the sera of allergic patients.

The amino acid sequence of Che a 1 displays striking differences with those of the members of the Ole e 1-like protein family. This supports the previously suggested idea that only those members of this family belonging to very taxonomically related species, such as species of the Oleaceae family (olive, lilac and privet), exhibit high amino acid sequence identities (higher than 80%) [21, 22, 25, 26]. The chenopod allergen shows a single N-glycosylation site at Asn-39, which is only coincident with that of the homologous protein from tomato (*Lycopersicon esculentum*). On the basis of the positive reaction of Che a 1 with the lectin Con A, this position of the polypeptide sequence should be occupied by a sugar. Che a 1 also displays different processing sites from other members of the family at both the N- and C-terminal ends.

It is well known that most of the amino acid substitutions between homologous proteins appear on the surface of the molecule and, thus, differences in the antigenicity of the counterparts would mainly result from such changes. The limited similarity on the amino acid sequence between Che a 1 and Ole e 1 (30% identity) could explain the low IgE and IgG cross-reactivities found between these allergens. On the other hand, the presence of glycan moieties in both allergens could be responsible to some extent for the low but detectable IgE cross-reactivity between olive and chenopod allergens, since rOle e 1, which lacks the natural oligosaccharide present in nOle e 1, did not reach the inhibition level on IgE binding to Che a 1 as nOle e 1 did (fig. 6).

Although a low but measurable degree of cross-reactivity was found between Che a 1 and Ole e 1, the IgE binding of the allergic sera to Che a 1 did not correlate with the sensitivity to Ole e 1, overthrowing our preliminary hypothesis. The absence of correlation could be due to the limited sequence identity between these proteins, which would explain the antigenic dissimilarity of their IgE epitopes. It is interesting to note that Ole e 1 – together with its relatives Lig v 1 and Syr v 1 – Lol p 11, Pla l 1 and Che a 1 reported here belong to the Ole e1-like protein family. These proteins have been structurally and immunologically analyzed, and all of them have been found to be allergens [22, 25–28]. They exhibit 27–45% amino acid sequence identity, but scarce or no antigenic equivalence has been reported for them. However, they presumably have an equivalent biochemical function in their corresponding natural sources. Since the hypothesis of the putative role of the biological activity of allergens in the mechanism of inducing allergy is an open question [29],

this family of allergenic proteins could be a good model for the study.

Finally, and taking into account the conservation among the amino acid sequences of Ole e 1-like proteins from related species, an implication of Che a 1 in the observed IgE cross-reactivity [3, 8, 9] between pollens from the Chenopodiaceae family, and even from the Amaranthaceae family, cannot be discarded.

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