

Cloning and Expression of *Ole e I*, the Major Allergen from Olive Tree Pollen

POLYMORPHISM ANALYSIS AND TISSUE SPECIFICITY*

(Received for publication, December 21, 1993, and in revised form, February 24, 1994)

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Ole e I, the major allergen from the olive tree (*Olea europaea*), is one of the main causes of allergy in Mediterranean countries and some areas of North America. The cloning and sequencing of several cDNAs coding for the olive allergen have been achieved. cDNA has been synthesized from total pollen RNA and amplified by using the polymerase chain reaction. The nucleotide sequence data demonstrate the existence of microheterogeneities in at least 37 positions out of the 145 amino acids of *Ole e I*, thus explaining the high degree of polymorphism exhibited by the natural protein. One of the sequenced cDNAs encoding a full-length isoform was inserted into the plasmid vector pGEX-2T and overexpressed. The recombinant *Ole e I* has been produced in *Escherichia coli* as a fusion protein with glutathione S-transferase of *Schistosoma japonicum*. This chimeric protein was purified by affinity chromatography on a glutathione-Sepharose 4B column and digested with thrombin to release the recombinant allergen. Both the fusion protein and the recombinant *Ole e I* were recognized in Western blot analysis by rabbit polyclonal and mouse monoclonal antisera raised against native *Ole e I* as well as by the IgE of olive pollen-sensitive human sera. This indicates that the recombinant production of individual isoforms may be useful for the improvement of reagents to be used in diagnosis and therapy of IgE-mediated disorders. In addition, *Ole e I* mRNA has been observed to be pollen-specific as shown in a Northern blot analysis.

Type I allergy is a clinical disorder that affects more than 15% of the human population in developed countries. The isolation and characterization of proteins responsible for IgE-mediated allergies (allergens) have been a main goal of research in the last few years. Allergens are usually proteins of molecular mass in the range of 8–50 kDa exhibiting particular features in terms of solubility and stability, many of them being capable of crossing the gut or respiratory barriers and triggering the IgE-mediated reactions.

Mites and both grass and tree pollens are considered the main natural sources producing allergy by inhalation. Among them, olive pollen allergy is a major human health problem in the Mediterranean area (1, 2). More than 70% of olive-allergic patients are sensitive to *Ole e I*, the major allergen from *Olea europaea* pollen (3). This protein has been isolated, purified to homogeneity, and characterized by biochemical methods, including the determination of its amino acid sequence (4, 5). *Ole e I* is an acidic protein, that exhibits two variants of the same 145-residue polypeptide chain, glycosylated (20 kDa) and non-glycosylated (18.5 kDa) (6), and shows microheterogeneity at several positions of its primary structure (5). The biological role of *Ole e I* in the olive pollen is unknown. In this regard, it is remarkable that *Ole e I* shows sequence similarity with other proteins from pollens of nonrelated species. Its amino acid sequence exhibits 36 and 38% identity with the deduced sequences of the polypeptides encoded by the *LAT52* gene from tomato and the *Zmc13* gene from maize pollens, respectively (5). The proteins encoded by these genes seem to play key roles in pollen maturation, germination, and/or pollen tube growth (7, 8). These facts may suggest the potential biological function performed by *Ole e I* in the pollen grain.

Diagnosis and treatment of IgE-mediated allergy disorders require the production of large amounts of pure and well defined proteins. The use of recombinant DNA technology has proved to be a convenient tool to obtain homogeneous protein preparations, which are remarkably useful in the case of polypeptides as the allergens exhibit high sequence microheterogeneity. This approach will also allow the identification of the most important residues involved in the immunological response. Recently, the full-length cDNAs of many important allergens have been obtained and analyzed, including those from pollens of ragweeds (9–12), grasses (13–15), and trees (16–19). However, there is a complete lack of data on cDNA sequences of the Oleaceae allergens, and only scarce data are available on the recombinant production of pollen allergens in high yields (20, 21).

In the study herein presented, the polymorphism of the sequence of cDNA encoding the major olive allergen, as well as the tissue specificity of its mRNA, has been analyzed. In addition, an immunologically active and soluble recombinant *Ole e I* allergen has been produced in *Escherichia coli*.

MATERIALS AND METHODS

Isolation of *Ole e I* Allergen—Native *Ole e I* allergen was purified from olive (*O. europaea*) pollen (Allergon AB) as reported (5).

Isolation of Total RNA and cDNA Synthesis—Total RNA was isolated from olive pollen according to Ullrich *et al.* (22) with minor modifications. Pollen (0.5 g) was homogenized in a Polytron homogenizer (Brinkmann Instruments) in 0.1 M Tris-HCl, pH 7.5, containing 4 M guanidine thiocyanate, 0.5% sodium *N*-lauroylsarcosine (Fluka Chemie AG), 0.1%

* This work was supported by Grants PB89/087 and PB92/0195 from the Dirección General de Investigación Científica y Técnica to the Ministerio de Educación y Ciencia, Spain. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) X76395, X76396, and X76397.

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antifoam (Sigma), and 0.14 M 2-mercaptoethanol. The clear supernatant was centrifuged at a 5.7 M CsCl cushion in 0.01 M EDTA at 40,000 rpm on an SW 60 rotor (Beckman) for 12 h (23). The same procedure was carried out for the isolation of total RNA from different olive tree tissues (stem, leaf, and olive fruit), but an initial step of crushing in a mortar with liquid nitrogen was included. Double-stranded cDNA was synthesized from 5 µg of total RNA by using a cDNA synthesis kit (Clontech) and oligo(dT) as primer.

PCR-based Cloning of Olive Allergen cDNA—The oligonucleotide primers were designed based on the amino acid sequence of *Ole e I* (5). They were synthesized on an Applied Biosystems DNA synthesizer model 381A according to standard procedures (23). The sequences and descriptions of the oligonucleotides are shown in Table I. The cDNA pool (5 µl) that served as a template and the primers OL-1 and OL-2 were dissolved in PCR standard mixture. After a denaturation step at 95 °C for 15 min, the sample was subjected to 30 cycles of denaturation at 94 °C (60 s), annealing at 42 °C (90 s), and extension at 72 °C (120 s) in a Techne Thermal Cycler PHC-3 (New Brunswick Scientific) with *Taq* DNA polymerase (U. S. Biochemical Corp.). The agarose gel scissored band was reamplified by using 5 cycles of denaturation at 94 °C (60 s), annealing at 47 °C (60 s), chain extension at 72 °C (90 s), and 20 cycles at 94 °C (60 s), 55 °C (90 s), and 72 °C (90 s). Each amplification round was terminated by holding at 72 °C for 10 min and gradually cooled to room temperature. The fragment was purified by using the Magic PCR Prep kit (Promega), phosphorylated with T4 polynucleotide kinase (U. S. Biochemical Corp.), and inserted into the *Sma*I site of a blunt-ended phosphorylated pUC18 plasmid. The transformation of DH5αF'-competent cells and recombinant selection were achieved by standard procedures.

The cDNA fragment, amplified with primers OL-3 and OL-4 by using the above PCR procedure, was digested with *Eco*RI and *Bam*HI and incorporated into an *Eco*RI/*Bam*HI-digested pUC18 plasmid vector. This was used for transformation and selection of recombinants containing a cDNA encoding the full-length amino acid sequence of *Ole e I*.

Sequence Analysis of *Ole e I* cDNAs—DNAs from several pUC18 clones were sequenced by using the dideoxy chain termination method (24) with the Sequenase kit (U. S. Biochemical Corp.) and α-³⁵S-dATP. Sequencing primers for both strands, M13mp18 universal and reverse, were purchased from New England Biolabs. The sequencing was carried out according to the manufacturer's recommendations.

Northern Blot—Total RNA (5 µg) from different tissues (pollen, stem, leaf, and fruit) were fractionated by electrophoresis in 1.2% formaldehyde-agarose gel and blotted onto Hybond-N nylon membranes (Amersham Corp.) by using standard methods (23). Membranes were prehybridized with 50% formamide, 4 × saline/sodium/phosphate/EDTA buffer, 5 × Denhardt's solution, 0.5% SDS, and 0.1 mg/ml denatured herring sperm DNA. A 0.4-kilobase fragment, generated from cDNA by PCR with OL-1 and OL-2 primers, was radiolabeled with a commercial random-primed kit (Boehringer Mannheim) with [α-³²P]dCTP (3,000 Ci/mmol, Amersham Corp.). This probe was denatured and hybridized to the immobilized RNA in prehybridization solution for 16 h at 42 °C. Before autoradiography, blots were washed with 2 × SSC buffer, 0.1% SDS three times at room temperature and twice at 68 °C. The filter was exposed to Kodak x-ray film at -80 °C for 2 h.

Construction of Expression Plasmid—After sequencing, the cDNA clone OLE3c, whose deduced amino acid sequence was closest to that obtained by Edman degradation of *Ole e I*, was subcloned into the *Bam*HI/*Eco*RI sites of a pGEX-2T expression vector. Production of the recombinant protein was performed after transforming DH5αF' cells with the pGEX-2T/OLE3c plasmid. This construction encodes a fusion protein (GST-OLE3c) consisting of glutathione S-transferase from *Schistosoma japonicum* and a 6-amino acid peptide (Leu-Val-Pro-Arg-Gly-Ser, a thrombin cleavage site) followed by the *Ole e I* polypeptide chain.

Production and Purification of Recombinant Fusion Protein and Thrombin Treatment—Overnight cultures of DH5αF' cells containing the recombinant plasmid pGEX-2T/OLE3c were diluted 10-fold with Luria broth medium containing 0.1 mg/ml ampicillin. Cells were grown up to an absorbance value at 600 nm of 0.6. After induction with 1 mM isopropyl β-D-thiogalactoside (Sigma), cultures were maintained for 4 h and harvested by centrifugation at 4,000 × g for 20 min at 4 °C. The pellet was washed with PBS (10 mM sodium phosphate, pH 7.2, 140 mM NaCl, 2.7 mM KCl), resuspended in PBS containing 1% Triton X-100 and 1 mM phenylmethanesulfonyl fluoride, and mildly sonicated. The precipitated material was dissolved in 0.1 M glycine/NaOH, pH 9.0, con-



FIG. 1. Northern blot analysis of RNA from olive tree tissues. Five µg of total RNA from different tissues was electrophoresed in a 1.2% formaldehyde-agarose gel, transferred to a nylon membrane, and probed with radiolabeled cDNA encoding olive pollen allergen. Lane 1, olive fruit; lane 2, leaf; lane 3, stem; lane 4, pollen. Numbers correspond to kilobases of RNA standards (Boehringer Mannheim).

taining 8 M urea and fractionated by spin column chromatography on Sephadex G-25 (Pharmacia Biotech Inc.) equilibrated in 0.1 M glycine/NaOH, pH 9.0. The eluted protein was stored overnight at 4 °C. This fraction was applied onto a column (5 ml) of glutathione-Sepharose 4B equilibrated in PBS, 1% Triton X-100 and eluted with 10 mM reduced glutathione in 50 mM Tris-HCl, pH 8.0. Fractions containing GST-OLE3c fusion protein were dialyzed against 10 mM Tris-HCl, pH 8.0, and lyophilized. The fusion protein in 50 mM Tris-HCl, pH 8.0, containing 5 mM EDTA was digested with 1 NIH unit of bovine thrombin (Pierce Chemical Co.)/mg of protein at room temperature for 6 h.

Electrophoresis and Immunoblotting—Native and recombinant samples were analyzed by SDS-polyacrylamide gel electrophoresis according to Laemmli (25) in 15% polyacrylamide gels under reducing conditions. Proteins were visualized by Coomassie Brilliant Blue staining or electrophoretically transferred onto Immobilon membranes (Millipore) (26) for immunostaining as described (5, 6). For IgE binding analysis, the membranes were incubated overnight at 4 °C with a pool of olive pollen-allergic patient serum diluted 1:8 in PBS containing 3% skim milk and 0.1% Tween 20 and then incubated with horseradish peroxidase-labeled goat anti-human IgE antibodies (Nordic Immunological Laboratories) diluted 1:1,000 in PBS, 0.05% Tween 20, 1% skim milk (6). Alternatively, the membranes were incubated with rabbit polyclonal serum raised against *Ole e I* (diluted 1:5,000) or with *Ole e I*-specific mouse monoclonal antibody 19G6 (diluted 1:1,000). Goat anti-rabbit IgG (Bio-Rad) and goat anti-mouse IgG (Pierce Chemical Co.), both horseradish peroxidase-labeled, were used as the secondary antibody, respectively. The peroxidase reaction was developed alternatively with 3,3'-diaminobenzidine HCl, 0.03% H₂O₂ in PBS for 1 min or with the ECL Western blotting reagent (Amersham Corp.) as described (5, 6).

RESULTS

Isolation and Tissue Specificity of *Ole e I* mRNA—Northern blot analyses were conducted on total RNA purified from pollen, leaf, stem, and fruit tissues of olive tree. These samples were fractionated on formaldehyde-agarose gels, transferred onto nylon membranes, and hybridized with a radiolabeled probe. The ³²P-labeled PCR-amplified cDNA fragment, obtained with OL-1 and OL-2 primers and corresponding to the 408 bp between residues 9 and 145 of the protein, was used as probe. An abundant single 0.8-kilobase transcript was only detected in the pollen tissue since no detectable signal was observed in leaf, fruit, or stem tissues (Fig. 1). Thus, the *Ole e I* allergen is exclusively expressed in the pollen tissue.

Characterization of PCR-amplified Clones Coding for *Ole e I*—Total RNA from olive pollen was used to synthesize and amplify the *Ole e I* cDNA. Primers corresponding to the 5' and 3' ends of the sequence encoding the olive allergen cDNA clone

¹ The abbreviations used are: PCR, polymerase chain reaction; PBS, phosphate-buffered saline; bp, base pair(s).

were designed based on the amino acid sequence of the native protein (5). The use of degenerate primers covering the length of the protein provided very poor yields, and primer dimer products appeared. Therefore, two degenerate primers (OL-1 and OL-2; see Table I), which covered the region between the residue at position 9 and the COOH-terminal end of the protein (145th position), were used as an initial approach (Fig. 2a). The nucleotide sequences obtained from eight truncated cDNA clones are shown in Fig. 3. These results allowed both the analysis of the polymorphism of the allergen and the design of a non-degenerate primer complementary to its COOH-terminal end. Accordingly, degenerate and non-degenerate primers (OL-3 and OL-4, respectively; see Table I) were used for a second PCR amplification approach to obtain clones expressing a full-length allergen (Fig. 2b). OL-3 corresponds to the NH₂-terminal region (first 15 bp) of the protein, and OL-4 corresponds to the most abundant nucleotide sequence that matched the 3' end of the encoding sequence determined by the first approach. Three full-length cDNAs, OLE1c, OLE3c, and OLE5c, were obtained by this method. Their sequences are shown in Fig. 3. Both approaches provided cDNA fragments that migrated as homogeneous products in agarose gel electrophoresis (Fig. 2). The cDNA fragment corresponding to the full-length sequence (444 bp) would encode a polypeptide of 16–17 kDa. This value is slightly lower than that estimated for non-glycosylated *Ole e I* by SDS-polyacrylamide gel electrophoresis (18.5 kDa) (4), but it is in good agreement with the value obtained from mass spectrometry (16.6 kDa) (6) and the amino acid sequence of the allergen (16.3 kDa) (5).

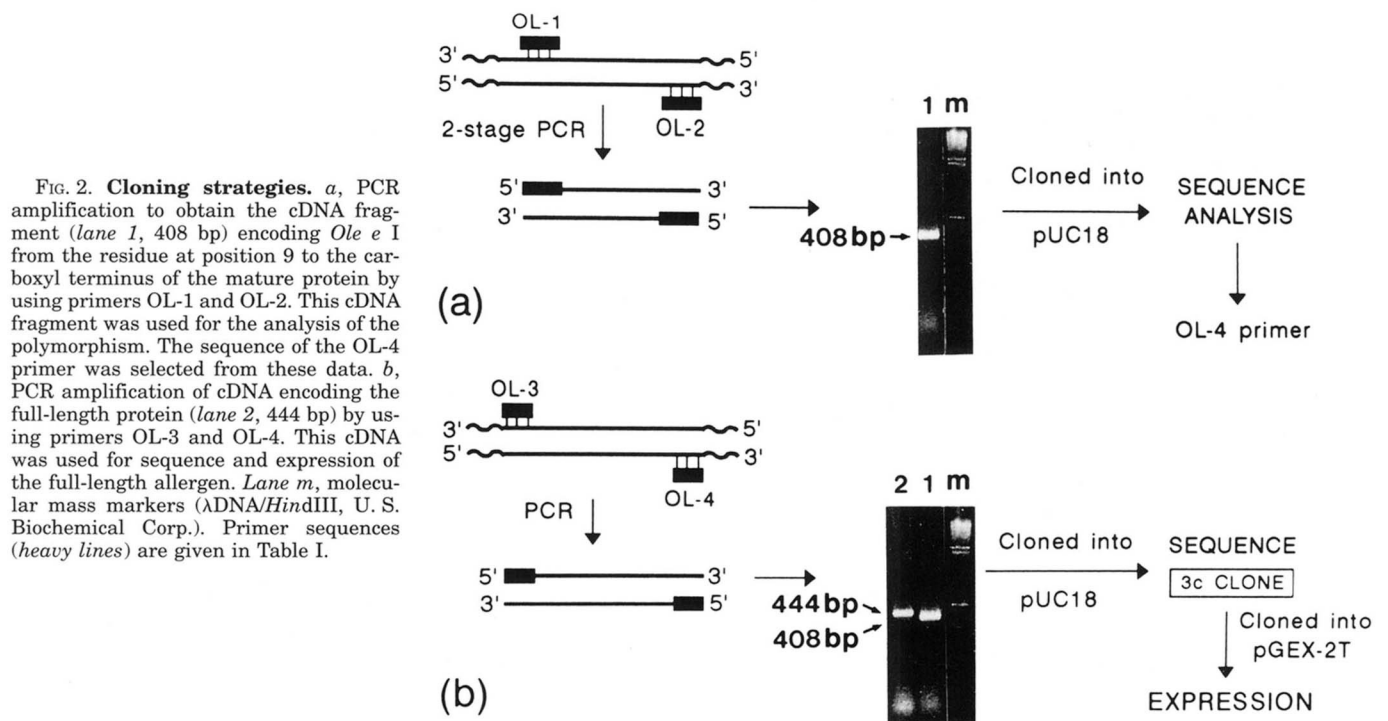
The amino acid sequences deduced from all of these cDNAs, partially or totally encoding the *Ole e I* allergen, are shown in Fig. 4 in comparison with the sequence obtained from the native protein by Edman degradation. Clones OLE16 and OLE20 code for the same amino acid sequence as it occurs in clones OLE33 and OLE37. A single amino acid exchange (99th position) is observed between the sequence deduced from clone OLE3c and that of the natural allergen. All of the sequences maintain the glycosylation site described for the protein at Asn-111. The differences obtained by comparing each of the nucleotide sequences, as well as their deduced amino acid sequences, are shown in Table II. Heterogeneity appears at 37 positions of the primary structure of the olive allergen (Fig. 4), but no more than 22 residues are different when deduced amino acid sequences are compared with each other. At least 85% identity exists between every pair of polypeptide sequences. The observed similarity is strongly increased if conservative changes such as Val/Ile/Leu, Arg/Lys, or Glu/Asp are considered. According to the IUIS nomenclature for allergens (27), the protein isoforms encoded by the full-length nucleotide sequences here reported, OLE3c, OLE5c, and OLE1c, can be called *Ole e I.2*, *Ole e I.3*, and *Ole e I.4*, respectively, since *Ole e I.1* is assumed for the main sequence obtained by Edman degradation.

Expression of *Ole e I*-encoding cDNA—The expression of the recombinant full-length *Ole e I* protein was performed in *E. coli*. The *Bam*HI/*Eco*RI fragment of OLE3c was selected to be subcloned and expressed in the pGEX-2T plasmid, which contains the glutathione *S*-transferase gene under the control of

TABLE I
Oligonucleotides used in PCR and sequencing

In the nucleotide sequence, *R* represents G/A, *Y* represents C/T, *Z* represents A/T/C, and *N* represents A/T/C/G. Numbers in parentheses correspond to the positions of the peptides in the primary structure of the allergen (5). The *Bam*HI and *Eco*RI restriction sites are underlined. OL-4 contains a translational stop codon at the 3' end of the sequence.

Oligomer	Nucleotide sequence	Strand	Amino acid sequence
OL-1	5'CARTTYCAYATZCARGGNCARGT3'	Coding	QFHIQGQ (9–15)
OL-2	5'ATCATRTTNGGNGGRTACATNCC3'	Noncoding	MYPPNM (140–145)
OL-3	5'CCGGGATCCGARGAYGTNCCNCA3'	Coding	EDVPQ (1–5)
OL-4	5'CGGAATTCTCACATGTTGGGCGGGTA3'	Noncoding	YPPNM (141–145)



the *tac* promoter (28). This construction would produce a fusion protein composed of glutathione *S*-transferase and the recombinant allergen linked through a hexapeptide that contains a cleavage site for the proteolytic activity of thrombin. The expression of the inserted cDNA fragment generated a recombinant protein, GST-OLE3c, with an estimated molecular mass of 42 kDa (Fig. 5), which agrees with the molecular mass of 26 kDa for glutathione *S*-transferase (29) and 16.3 kDa for the polypeptide chain of *Ole e* I (5). Although the yield was about 100 mg of fusion protein/liter of cell culture, less than 2% of this protein remained soluble after lytic treatment of the transformed cells (Fig. 5). After treatment of the pellet in 8 M urea, spin column separation, and folding overnight (30), the soluble material was chromatographed over a glutathione-Sepharose affinity column. The eluted GST-OLE3c protein fusion (about 25 mg/liter of cell culture) (Fig. 5) was digested with thrombin. The molecular mass of the recombinant *Ole e* I obtained by the proteolytic treatment agrees well with that of the polypeptide chain of the olive allergen (Fig. 5). As a consequence of the cloning, recombinant *Ole e* I should contain 2 extra residues (Gly-Ser) at the amino terminus.

The thrombin cleavage product as well as the GST-OLE3c fusion protein were used for immunological assays. After Western blotting and immunostaining, GST-OLE3c and recombinant *Ole e I* were recognized by a rabbit antiserum obtained against *Ole e I* and by the *Ole e I*-specific monoclonal antibody 19G6 (Fig. 6, *a* and *b*). These data demonstrate the existence of B-cell epitopes shared by the native and the recombinant olive pollen allergen. Finally, IgE antibodies from sera of olive pollen-allergic patients bind in Western blot analyses to both recombinant *Ole e I* and GST-OLE3c fusion protein (Fig. 6*c*), indicating the presence of allergenic epitopes in the expressed molecules.

A simple and efficient method has been employed to clone olive pollen cDNA molecules, which encode the translated region of the main allergen *Ole e 1* in *O. europaea*. This method avoids the tedious screening of a cDNA library. By using the PCR, eight partial- and three full-length distinct olive allergen cDNA clones have been synthesized and sequenced. The degree and nature of the protein polymorphism were evaluated mainly with the former sequences. With a full-length open reading frame nucleotide sequence, a construction was designed to achieve the expression of the recombinant allergen.

There are two segments in the deduced sequences from po-

FIG. 3. Nucleotide sequences of olive pollen allergen (truncated and full-length isoforms) cDNAs. Numbering begins at the GAG codon corresponding to the NH₂-terminal residue (Glu) of the natural protein. Dashes indicate identity of nucleotides with respect to the upper sequence.

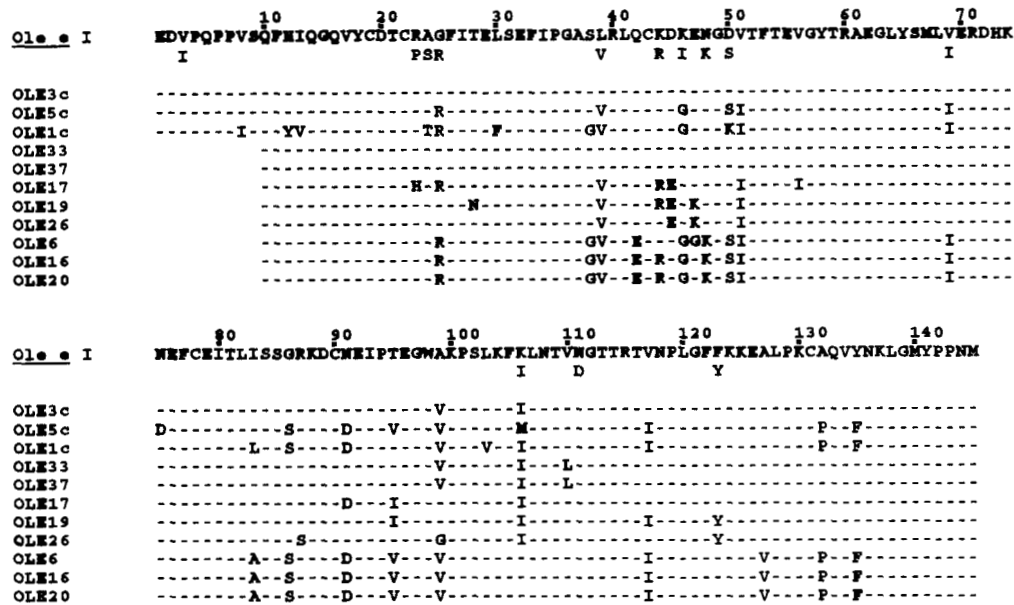


FIG. 4. Deduced amino acid sequences of the obtained cDNAs encoding *Ole e I*. Sequences are shown in comparison with that obtained by Edman degradation of the purified *Ole e I* (5). Microheterogeneities detected in the natural protein (5) are shown below the main sequence. Dashes indicate identity with respect to the main sequence of *Ole e I*.

TABLE II
Comparison of nucleotide and deduced amino acid sequences of olive pollen allergen cDNAs

The number of differences among pairs of nucleotide and amino acid sequences are shown in the upper right and lower left sections, respectively.

	OLE3c	OLE33	OLE37	OLE26	OLE19	OLE17	OLE6	OLE16	OLE20	OLE5c	OLE1c
OLE3c		3	7	27	30	27	52	51	51	45	55
OLE33	1		4	30	29	28	53	54	54	48	56
OLE37	1	0		29	28	26	54	51	51	50	58
OLE26	7	8	8		25	31	61	60	60	52	64
OLE19	10	11	11	6		34	59	59	59	56	63
OLE17	10	11	11	10	8		50	47	47	45	53
OLE6	19	20	20	21	21	20		7	7	35	51
OLE16	19	20	20	22	20	18	2		0	34	54
OLE20	19	20	20	22	20	18	2	0		34	54
OLE5c	14	15	15	17	17	15	8	8	8		44
OLE1c	18	19	19	21	21	19	12	13	13	11	

sitions 38 to 51 and 83 to 99 of the olive pollen allergen where the microheterogeneity is mainly located. These regions are predicted by theoretical approaches as the most hydrophilic and antigenic areas of *Ole e I* (5). These observations enhance the importance of the immunological study of the individual isoforms to allow the mapping of the allergen epitopes. The cloning and expression of specific clones seem to be the most available approach to obtain well defined and homogeneous *Ole e I* molecules, since the isolation and purification of each isoform protein are in practice very difficult (4) due to the high number of variants and their close similarity.

A high degree of polymorphism is characteristic of plant pollen allergens. Among other examples, grass pollen allergens such as *Lol p II* and *Lol p III* exist in multiple isoallergenic forms, which are variants that appear to be immunologically indistinguishable (32). The polymorphism of short ragweed pollen allergens, *Amb a I* proteins, which are products of a multigene family (33), has even been analyzed on mRNA isolated from the flower of a single plant (34), demonstrating that each individual plant can express multiple forms of the allergen in the pollen grains. Whether this polymorphism stands for differences in the functional features of these proteins is yet unknown, but it has been demonstrated that few changes in the sequence of an allergen can be detected by individual sera of hypersensitive patients (19).

cDNA cloning and expression of allergens should provide information regarding their structural and functional features

and elicit new data about how these allergens promote the IgE response in affected individuals. We have successfully expressed the olive pollen allergen as a fusion protein composed of glutathione *S*-transferase and the complete sequence of *Ole e I*. The soluble protein is produced in a high yield. It seems that expression as fusion proteins would be an efficient way of obtaining recombinant allergens since *Amb a V* and *Amb t V*, obtained by a similar construction as the one herein employed (20), and *Lol p II*, obtained as a fusion protein with human ferritin H-chain (21), have recently been produced with high recoveries. The recombinant *Ole e I* allergen retained its ability to bind IgE from the sera of allergic patients and is also recognized by *Ole e I*-specific polyclonal and monoclonal antibodies. This indicates that recombinant and natural *Ole e I* share IgE and IgG determinants. Therefore, the expression at a level of milligrams of well defined and immunologically active *Ole e I* allergen will allow future studies on the identification of B- and T-cell epitopes.

RNA blot hybridization analyses demonstrate that the transcript of this protein is localized exclusively in the pollen tissue and not in other tissues such as leaf, stem, and fruit. This could explain the absence of hypersensitivity of olive pollen-allergic patients when they ingest olive fruit or olive oil. Pollen-specific localization was also reported for the genes *LAT52* from tomato (7) and *Zmc13* from maize (8). The sequences of the proteins encoded by these genes exhibit 36 and 38% sequence identity with *Ole e I*, respectively, and no gaps were included for the

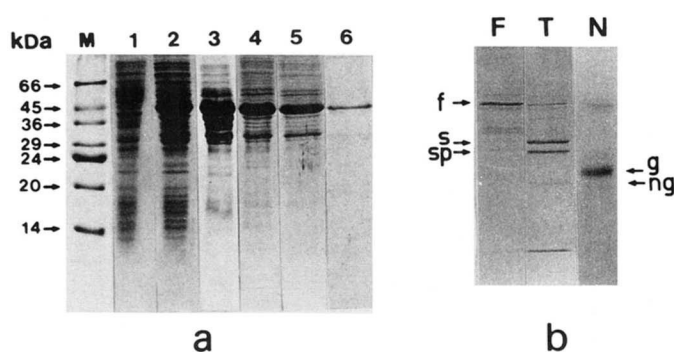


FIG. 5. SDS-polyacrylamide gel electrophoresis (15% acrylamide under reducing conditions) analysis of the purification procedure (a) and thrombin treatment (b) of the recombinant GST-Ole e I fusion protein. a: lanes 1 and 2, total cell lysate from *E. coli* strain without and with pGEX-2T/OLE3c insert, respectively; lane 3, insoluble fraction of sample on lane 2; lane 4, sample of lane 3 after solubilization in 8 M urea; lane 5, sample of lane 4 after elution from G-25 spin column; lane 6, GST-OLE3c eluted from the glutathione-Sepharose 4B column; M, molecular mass markers (Bio-Rad). b: lane F, GST-OLE3c fusion protein; lane T, GST-OLE3c treated with thrombin; lane N, natural Ole e I. The arrows indicate the mobility of the GST-OLE3c fusion protein (f), glutathione S-transferase (s), glutathione S-transferase thrombin product (sp), and glycosylated (g) or non-glycosylated (ng) Ole e I.

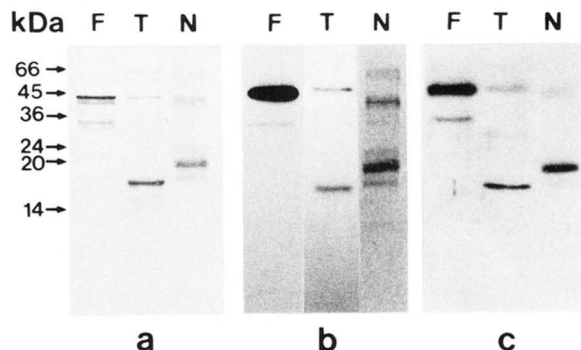


FIG. 6. Western blotting of the recombinant proteins. Lanes F, T, and N are as shown in Fig. 5b. Membranes were immunostained with the following: a, Ole e I-specific rabbit polyclonal antiserum; b, Ole e I-specific mouse monoclonal antibody 19G6; and c, olive pollen allergen-hypersensitive human sera. Molecular mass (kDa) markers are included.

alignment of their amino acid sequences (5). The polypeptide products of these genes seem to be involved in some event in pollen germination (7, 8). Therefore, although the *in vivo* function of Ole e I remains uncertain, both its specific tissue localization and the mentioned significant sequence similarity may suggest that this allergen could be involved in germination.

In conclusion, the cDNA nucleotide sequences herein reported establish the existence of multiple mRNA molecules in the olive tree pollen that code for allergen isoforms. One of these cDNAs has been expressed in *E. coli*, producing a high yield of soluble fusion protein, which was digested with thrombin, releasing the recombinant allergen. Both the fusion protein and the recombinant Ole e I share common immunological properties with the natural allergen. In addition, the expression in *E. coli* of specific clones of the Ole e I allergen offers the possibility of obtaining well defined and homogeneous olive allergen molecules to be used for research, clinical diagnosis,

and therapy of this IgE-mediated disorder. The functional analysis of pollen-specific Ole e I-like proteins could also be performed with expressed cDNA clones. Finally, the employed expression strategy may be of general interest for producing large amounts of recombinant allergens in *E. coli*.

Acknowledgments—We are grateful to Drs. J. G. Gavilanes and A. Martínez de Pozo for critical reading of the manuscript and Dr. C. López-Otín for oligonucleotide synthesis.

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OCR

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Con correzioni manuali

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ole e 1

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