

Molecular cloning and expression of active Ole e 3, a major allergen from olive-tree pollen and member of a novel family of Ca^{2+} -binding proteins (polcalcins) involved in allergy

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A cDNA encoding Ole e 3, a major allergen from olive-tree pollen, has been cloned and sequenced. A strategy based on two-step PCR amplification towards the 5' end and 3' end, with an internal specific primer, has been used. The isolated cDNA contains an open reading frame coding for a polypeptide of 84 amino acids, which is in agreement with the composition and molecular mass of the natural allergen, exhibiting two 12-residue segments homologous to Ca^{2+} -binding sites of EF-hand type. The cDNA was inserted into the pET-11b expression vector and over-expressed in *Escherichia coli*. The purified recombinant protein shows identical secondary structure to that of the natural allergen and is able to bind both IgE from sera of patients allergic to olive pollen and polyclonal antibodies raised against olive-pollen Ole e 3. The capacity of binding Ca^{2+} has been demonstrated for both natural and recombinant allergens. RNA transcripts of Ole e 3 were only detected in pollen tissue. Northern-blot and Western-blot analyses of poly(A)⁺RNA and protein extracts, respectively, obtained from a variety of olive-tree-related and non-related mature pollens demonstrated the presence of Ole e 3 homologous proteins. This indicates a sequence conservation and widespread distribution for this family of Ca^{2+} -binding proteins that can be responsible for allergenic cross-reactivity. We suggest the tentative generic name of polcalcins for the members of this family of Ca^{2+} -binding proteins from pollen.

Keywords: allergen; Ole e 3; recombinant allergen; olive-pollen allergy; Ca^{2+} -binding proteins; nucleotide sequence.

Pollinosis is an IgE-mediated allergic process induced by airborne pollen grains. It frequently depends on the botanical behaviour surrounding the affected individual, since the allergenic pollens are anemophilous and should reach organisms by scattering on the air. Wild grasses – *Lolium perenne*, *Cynodon dactylon*, *Phleum pratense* – and woods – *Ambrosia artemisiifolia*, *Ambrosia vulgaris* – are widespread vegetable sources causing pollinosis. However, some trees such as birch and olive are able to induce dramatic airborne allergies because of seasonal appearance of high-density pollen levels. In fact, allergy to olive-tree (*Olea europaea*) pollen affects more than 30% of the population in Mediterranean areas during the pollination season [1]. More than 70% of the patients allergic to olive pollen are sensitive to Ole e 1 [2], one major allergen from this pollen [3, 4]. Recently, a novel olive-pollen allergen, Ole e 3 (9.2 kDa), has been isolated and partially characterised [5]. Its prevalence accounts for more than 50% of the olive allergic patients, depending on the geographical area, and is thus considered as a major allergen [6]. Partial amino acid sequence data of Ole e 3 revealed the

presence of at least one Ca^{2+} -binding domain similar to those of calmodulin and parvalbumin [5]. It is well known that pollen germination and pollen-tube growth are strongly influenced by both distribution and intracellular levels of Ca^{2+} , the metabolism of which is controlled by calmodulin-related proteins [7–10]. However, the identification and characterisation of Ca^{2+} -binding pollen proteins is very scarce. A few Ca^{2+} -binding proteins have been described among pollen proteins: Bet v 3 displaying four Ca^{2+} -binding sites in its 26-kDa polypeptide chain [11], and Bra r II, Cyn d 7 and Bet v 4, with two sites in their 9-kDa chains [12–14]. These pollen proteins have been characterised as allergens, their binding to IgE antibodies of allergic patients sera being dependent on the presence of Ca^{2+} [12–15].

We herein report the molecular cloning and expression of Ole e 3 in *Escherichia coli*, as well as its purification and characterisation. In addition, we also demonstrate that Ole e 3 binds Ca^{2+} and is specifically expressed in pollen. The involvement of Ole e 3 in allergenic cross-reactions is also discussed.

MATERIALS AND METHODS

cDNA synthesis. ds cDNA was synthesised from 50 µg total RNA, isolated from olive pollen as described [4], using a modified lock-docking oligo-dT primer for the synthesis of the first strand. After creation of blunt-ends with T4 DNA polymerase, the ds cDNA was ligated to an adaptor sequence. All these reactions were performed following the protocol [16] of the Marathon cDNA amplification kit (Clontech).

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Abbreviations. rOle e 3, recombinant Ole e 3; nOle e 3, natural Ole e 3; AP1 sense primer.

Note. The novel nucleotide sequence reported here has been submitted to the GenBank/EMBL database and is available under accession number AF015810.

PCR amplification and cloning of olive-allergen cDNA. PCR amplifications were performed via a two-step protocol. A gene-specific antisense oligonucleotide (OL3-1: 5'-TCGGTTCRATCTCRGCCATCAT-3') was designed, based on the internal peptide MMAEIDTD of the purified nOle e 3 [5]. A Marathon adaptor primer, which hybridises with the adaptor sequence, was used as a sense primer (AP1: 5'-CCATCCTAA-TACGACTCACTATAGGGC-3'). PCR amplification was carried out on a Perkin-Elmer Gene Amp System 2400. The PCR conditions and cycle numbers employed are described in the user manual kit, using the Advantage KlenTaq Polymerase Mix (Clontech). After this step, a single PCR product of about 300 bp was visualised on a 2% agarose gel, and purified using the GENO-BIND DNA purification protocol included in the Marathon kit. After phosphorylation with T4 polynucleotide kinase, the fragment was inserted into a *Sma*I-site-dephosphorylated pUC18 plasmid. The transformation of DH5 α F'-competent cells and recombinant selection were achieved by standard procedures.

The DNA nucleotide sequencing of several clones was automatically carried out using the AB1 PRISM kit, according to the dideoxy chain-termination method [17] adapted for PCR, and the employment of fluorescent-labelled primers (Perkin-Elmer) on an AB1 PRISM 377 DNA sequencer (Perkin-Elmer). In the second step, a non-degenerate oligonucleotide, based on the NH₂-terminal end of the protein (MADDPQE), deduced from the partial sequence obtained above, was used as a sense primer (OL3-2: 5'-ATACATATGCGCCGACGATCCACAGGA-3'). A *Nde*I restriction site (underlined) was introduced. The same AP1 primer was used in this amplification as antisense primer. PCR amplification was carried out as above and a single band of about 550 bp was obtained. This fragment was included into the same vector described above and further sequenced.

Northern blot. Total RNA (15 μ g) isolated from different pollen samples (olive, lilac, birch, Bermuda grass, ryegrass, mugwort and pine) was fractionated by electrophoresis in a 1.2% formaldehyde/agarose gel and blotted onto Hybond-N nylon membranes (Amersham) using standard methods [18]. The 165-bp fragment, generated from olive-pollen cDNA by means of PCR with OL3-1 and OL3-2 primers, was used as a probe, radiolabelled with a commercial random-primer kit (Boehringer Mannheim) containing [α -³²P]dCTP (6000 Ci/mmol). The hybridisation conditions were selected following the experimental procedure described [4].

Construction of expression plasmid. The sequenced cDNA included into *Sma*I-pUC18 (pUC-Ole3) was subcloned into the *Nde*I/*Eco*RI of a pET-11b expression vector (pET-Ole3). Production of the recombinant protein as a non-fusion molecule was performed after transforming BL21(DE3)-competent cells with pET-Ole3 construction.

Expression and purification of recombinant protein. Overnight cultures of BL21(DE3) cells containing the recombinant plasmid pET-Ole3 were diluted tenfold with Luria-Bertani medium containing 0.1 mg/ml ampicillin, and grown up to an absorbance value at 600 nm of 1.0. After induction with 0.4 mM isopropyl β -D-thiogalactoside (Sigma), cultures were maintained for 4 h and harvested by centrifugation at 4000 $\times$ g for 20 min at 4°C. The pellet was re-suspended in distilled water containing 1 mM phenylmethylsulfonyl fluoride, and lysed by two cycles of freezing and thawing (42°C). After centrifugation at 10 000 $\times$ g, at 4°C, the soluble fraction was lyophilised and chromatographed on a gel-filtration sephadex G-50 column equilibrated in 0.2 M ammonium bicarbonate. The fractions containing recombinant Ole e 3 (rOle e 3), detected with specific polyclonal antibodies against the natural allergen (nOle e 3), were lyophilised and chromatographed on a DEAE-cellulose column

with a gradient (20–500 mM) of ammonium bicarbonate, pH 8.0.

Electrophoresis and immunological assays. nOle e 3 and rOle e 3 were analysed by SDS/PAGE according to Laemmli [19] in 15% polyacrylamide gels. Proteins were either visualised by Coomassie brilliant blue staining or electrophoretically transferred onto nitrocellulose membranes (Amersham) for immunodetection as described [4] using a pool of 15 sera from different olive-pollen-allergic patients (Rast 4–6), diluted 1:10, or a rabbit polyclonal antiserum raised against nOle e 3, diluted 1:5000. The signal was developed using the ECL Western-blotting reagent (Amersham). ELISA titration was performed as described [5]. After coating with 100 μ l antigen (1 μ g/ml), the plates were incubated with different dilutions of the pool of sera, followed by mouse anti-human IgE and horseradish-peroxidase-labelled goat anti-mouse IgG. Alternatively, the antigen-coated wells were incubated with different dilutions of anti-nOle e 3 rabbit antiserum, followed by horseradish peroxidase-labelled anti-rabbit IgG.

CD analysis. CD spectra were obtained on a Jasco J-715 spectropolarimeter fitted with a 150-W xenon lamp. Four spectra were accumulated in the far-ultraviolet region and recorded at 50 nm/min scanning speed. The sample was analysed in 0.1-cm optical-path cells and in 20 mM ammonium bicarbonate, pH 8.0, at 25°C. The protein concentration was in the range 0.20–0.25 mg/ml. Mean residue mass ellipticities were calculated based on 110.8 as the average molecular mass/residue, obtained from the amino acid composition, and expressed in terms of θ (degree $\cdot$ cm² $\cdot$ dmol⁻¹).

Ca²⁺-binding assay. Assay of Ca²⁺ binding was carried out according to [20] with minor modifications. 1 nmol nOle e 3, rOle e 3, parvalbumin (positive control) and lysozyme (negative control) were dotted onto a nitrocellulose membrane. After washing three times in calcium buffer (10 mM Pipes, pH 6.9, containing 50 mM NaCl, 0.1 mM MgCl₂), the membrane was incubated with 6 μ M ⁴⁵CaCl₂ (3000 mCi/mmol) in calcium buffer, and washed twice with distilled water. The membrane was exposed to Agfa X-ray film for 4 h.

Amino acid composition and sequence analysis. Protein samples (0.5–2.0 nmol) were hydrolysed with 5.7 M HCl at 105°C for 24 h as described [3]. Hydrolysed samples were analysed on a Beckman System 6300 amino acid analyser. NH₂-terminal Edman degradation of rOle e 3 (0.8 nmol) was performed on an Applied Biosystems model 477A sequencer as described [5]. Amino acid sequence alignments and searches for similarities were performed according to [21]. Sequence-based secondary structure was predicted according to [22].

RESULTS

Molecular cloning and nucleotide sequence of Ole e 3 allergen. A cDNA encoding Ole e 3, a major allergen from olive-tree pollen, was amplified by PCR. In a first step, both an antisense and degenerate oligonucleotide primer, designed according to the sequence of an internal peptide of nOle e 3 obtained by CNBr treatment of the protein [5], and a sense primer (AP1), complementary to the adaptor sequence ligated to the cDNA template in both sides, were used. In a second step, a sense non-degenerate primer and AP1 were used to amplify the complete sequence of this DNA. This DNA was used for cloning into a pUC18 plasmid (pUC-Ole3). From nucleotide sequence analyses, several clones were obtained, rendering a coding region of 252 nucleotides, which corresponds to a polypeptide chain of 84 amino acids (9360 Da calculated molecular mass) (Fig. 1).

The deduced amino acid sequence of Ole e 3 displays two potential Ca²⁺-binding sites at positions 19–30 and 54–65. Its

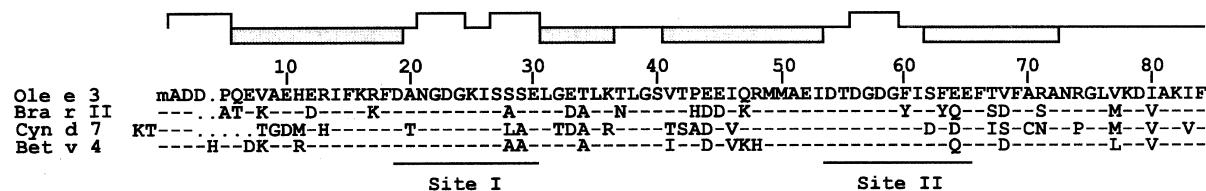


Fig. 1. Amino acid sequence of Ole e 3 and secondary structure prediction. Upper: Theoretical prediction of the secondary structure of Ole e 3: α -helix, shaded boxes; β -turns, upper line. Lower: Comparison of Ole e 3 amino acid sequence with the allergens Bra r II from rapeseed, Cyn d 7 from Bermuda grass, and Bet v 4 from birch pollens. Dashes indicate identities with respect to Ole e 3. EF-hand Ca^{2+} -binding sites are indicated by solid lines. Gaps are indicated by dots. Numbering is according to rOle e 3.

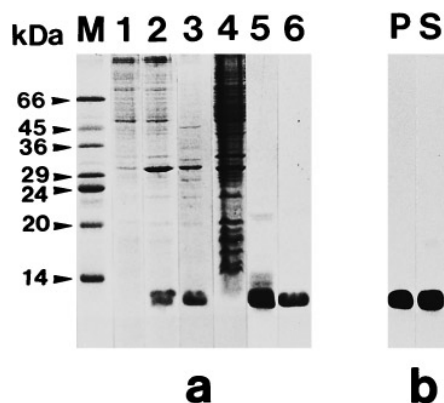


Fig. 2. Expression and purification of recombinant Ole e 3. (a) Coomassie blue-stained 15% SDS/PAGE under reducing conditions. Lane 1 and lane 2, crude extract from *E. coli* after 30 min and 4 h of induction with isopropyl- β -D-thiogalactoside, respectively; lane 3 and lane 4, soluble and insoluble fractions, respectively, of the total cell lysate; lane 5, eluate from sephadex G-50 column; Lane 6, purified rOle e 3 by elution from the DEAE-cellulose column; M, molecular-mass markers (Bio-Rad). (b) Western-blotting of the purified rOle e 3. Membranes were immunostained with nOle e 3-specific rabbit polyclonal antiserum (P) and olive-pollen-hypersensitive human sera (S).

pairwise comparison with those from Ca^{2+} -binding pollen allergens (Fig. 1) shows identities of 63% with Cyn d 7 from Bermuda grass, 74% with Bra r II from rapeseed and 81% with Bet v 4 from birch, and 85%, 91% and 95% similarities, respectively. These data suggest that they are members of a highly conserved family of pollen proteins.

Production of recombinant Ole e 3 in *E. coli* and characterisation. The complete coding sequence of Ole e 3 included in the construct pUC-Ole3 was uni-directionally subcloned into *NdeI/EcoRI*-pET11b expression vector, transformed in *E. coli* BL21(DE3) and its intracellular expression as a non-fusion protein was induced for 0, 0.5, 2 and 4 h (Fig. 2a). A band of 10 kDa was detected in the electrophoretic profile corresponding to the induced cells. The recombinant protein was found in the soluble fraction of the harvested and disrupted cells. It was purified by gel-filtration chromatography on sephadex G-50 and DEAE-cellulose ion-exchange column, rendering about 25 mg of soluble and active rOle e 3 per liter of cell culture.

Small differences are observed between the amino acid compositions of nOle e 3 and rOle e 3, which could be due to microheterogeneity of the natural protein. Edman degradation of rOle e 3 renders (NH₂-) ADDPQEV-, indicating the absence of methionine at the NH₂-terminal end of the polypeptide chain, which should be processed by the bacteria (Fig. 1).

The CD spectrum of rOle e 3 is given in Fig. 3. No differences are observed for either nOle e 3 or rOle e 3, along the far-

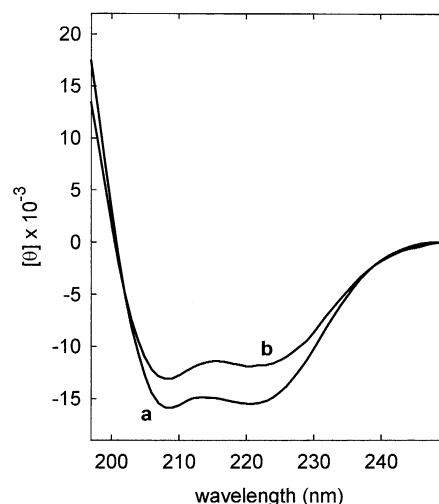


Fig. 3. Far-ultraviolet CD spectra of recombinant Ole e 3. The spectra obtained for rOle e 3 and nOle e 3 are coincident both in the absence (a) and in the presence (b) of 2 mM EGTA. The protein concentration was 27 μM . Ellipticity values are expressed in units of degree $\cdot \text{cm}^2 \cdot \text{dmol}^{-1}$.

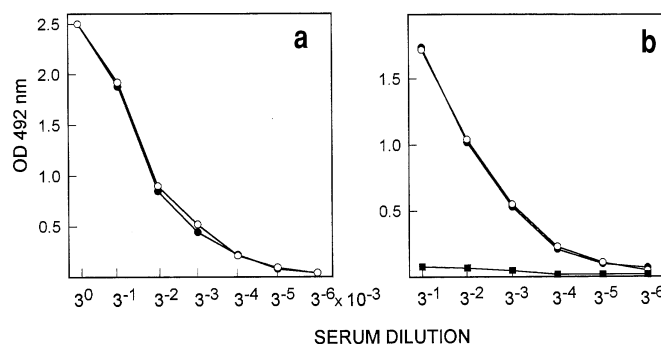


Fig. 4. IgG and IgE binding analysis of rOle e 3. ELISA titrations of wells coated with rOle e 3 (●) or nOle e 3 (○) by: (a) specific polyclonal rabbit antiserum raised against nOle e 3; (b) pool of sera from patients allergic to olive pollen. (■) A pool of sera from non-allergic individuals was used as control.

UV wavelength range, indicating identical secondary structure, 52% α helix and 10% β strand [5].

ELISA and immunoblotting analyses with nOle e 3-specific rabbit IgG antibodies as well as with sera from allergic patients have been performed to study the immunological properties of the recombinant protein. rOle e 3 behaves as the natural allergen in terms of recognition by both IgG and IgE antibodies after SDS/PAGE and transference to membranes (Fig. 2b), and when fixed to wells (Fig. 4). In addition, sera from non-allergic in-



Fig. 5. Ca^{2+} -binding activity of Ole e 3. Dot-blot assay of binding to $^{45}\text{Ca}^{2+}$ of natural (n) and recombinant (r) Ole e 3. The binding is compared with that from lysozyme (l) and parvalbumin (p) as negative and positive controls, respectively. 1 nmol/dot of protein (Ole e 3: 9.2 µg; lysozyme: 14.0 µg; parvalbumin 16.0 µg) was applied.

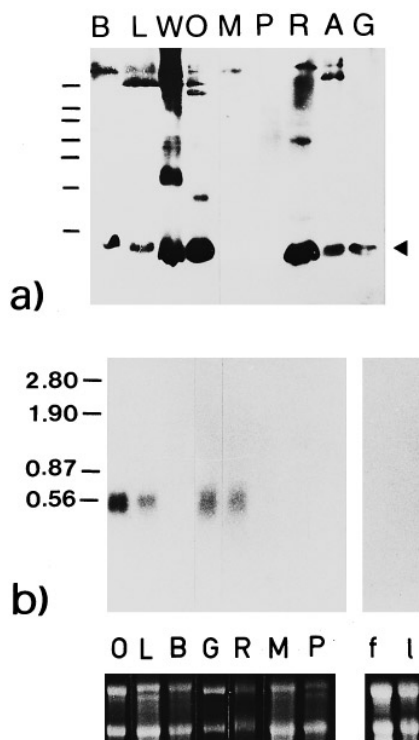


Fig. 6. Detection of Ole e 3-like proteins in pollens of different species, and tissues from olive tree. (a) Western analysis in SDS/PAGE under non-reducing conditions, of protein extracts obtained from pollen of birch (B), lilac (L), wheat (W), olive (O), mugwort (M), pine (P), ryegrass (R), ash (A) and Bermuda grass (G). Molecular-mass markers as in Fig. 2. The arrowhead indicates the mobility of Ole e 3. 50 µg total protein was applied in each lane. The immunostaining was achieved with Ole e 3-specific rabbit polyclonal antiserum. (b) Northern-blot analysis of RNA from: (O) olive, (L) lilac, (B) birch, (G) Bermuda grass, (R) ryegrass, (M) mugwort, and (P) pine pollens, as well from both olive tree (f) fruit and (l) leaves. 5 µg total RNA was probed with a radio-labelled cDNA segment encoding residues 1–56 of Ole e 3. The positions of RNA standards (kb) are indicated by bars. The lower panel shows the analysis of ribosomal RNA in order to assess the integrity of the samples.

dividuals were not reactive against these proteins. These results reveal that rOle e 3 retains the allergenic and antigenic epitopes of the natural allergen.

Binding of Ca^{2+} to natural and recombinant Ole e 3. Both nOle e 3 and rOle e 3 bind radioactive Ca^{2+} , and to a similar extent as parvalbumin, the positive control (Fig. 5). Moreover, the olive allergen is shown to contain bound Ca^{2+} , since its CD spectrum in the presence of 2 mM EGTA reveals a significant conformational change from that obtained in the absence of the chelant (Fig. 3), the α -helical content being decreased by 10%. The original conformation is recovered by addition of 5 mM CaCl_2 . rOle e 3 displays identical spectral changes. These results demonstrate that the conformation of Ole e 3 is Ca^{2+} dependent.

Detection of proteins homologous to Ole e 3 and tissue specificity. The potential presence of molecules displaying antigenic determinants similar to those of Ole e 3 allergen in the extracts of pollen from different plants, phylogenetically related and non-related to olive tree, has been shown by Western-blot immunostained with rabbit antiserum raised against nOle e 3. Positive protein bands of 9–10 kDa are observed in *Oleaceae*, *Gramineae*, and birch-pollen extracts (Fig. 6a). The different immunostaining intensities observed could be explained in terms of either a different protein concentration or a different degree of sequence similarity with Ole e 3. A 9.0-kDa protein band in the mugwort pollen extract was recognised after treatment with 2-mercaptoethanol and heating (data not shown). The immunostained high-molecular-mass protein bands observed for some pollen extracts may be related to polypeptide aggregates and were also detected during the purification of nOle e 3 [5].

Using an Ole e 3 cDNA fragment, RNA-blot studies were performed for total RNA purified from olive, lilac, mugwort, birch, ryegrass, Bermuda grass, and pine pollens, as well as from olive leaf and olive fruit. Northern-blot hybridisations were observed in *Oleaceae* and *Gramineae* pollens (Fig. 6b). No response was observed for either leaf or fruit olive tissues, indicating the specific expression of Ole e 3 in pollen.

DISCUSSION

A cDNA encoding Ole e 3, a major allergen from olive-tree pollen, has been cloned and sequenced. The amino acid sequence deduced from the open reading frame contains 84 residues and is in agreement with the molecular mass, amino acid composition and pI determined for the allergen isolated from olive-tree pollen [5]. The sequence of Ole e 3 is homologous to those of Bra n I and II, Cyn d 7 and Bet v 4 pollen allergens. Using a simple construction, Ole e 3 has been expressed and purified to give a high yield (25 mg purified protein/l *E. coli* culture), in contrast with the low yield obtained from the natural source (0.1 mg allergen/g olive-tree pollen). The Met residue located at the NH_2 -terminal end of the sequence deduced from the Ole e 3 cDNA is absent in the recombinant protein and in the natural allergen, as deduced from the amino acid composition of the corresponding tryptic peptide (data not shown). The recombinant protein shows the same structural and functional properties of the natural allergen. Therefore, the reported production of this recombinant allergen appears to be very suitable for clinical purposes, such as diagnosis and therapy.

Low polymorphism has been detected for cDNA encoding Ole e 3, since only one of the sequenced clones has leucine and valine at position 43 and position 80 instead of proline and isoleucine, respectively (Fig. 1). Microheterogeneity has also been detected by Edman degradation of peptides obtained from the natural protein (data not shown). This microheterogeneity could explain the slight differences between the amino acid compositions of rOle e 3 and nOle e 3. Polymorphism has also been described for the homologous allergens from *Brassicaceae* and Bermuda grass pollens [12, 13], thus suggesting that it is a common feature of this family of proteins, although to a much lesser extent than in other plant allergens, such as Ole e 1 [4] and Bet v 1 [23].

Natural and recombinant Ole e 3 show Ca^{2+} -binding activity, and they are shown to have bound Ca^{2+} , since the presence of EGTA disturbs their secondary structure. Two Ca^{2+} -binding sites are proposed to be present in Ole e 3, at position 19–30 (site I) and position 54–65 (site II). These amino acid sequences are highly similar to the 12-residue consensus sequence of the EF-

hand motifs of Ca^{2+} -binding proteins, such as parvalbumin or calmodulin. These two segments of Ole e 3 contain, at position 1 and position 6, the most conserved residues of the EF-hand domains, as well as the invariant Glu/Asp at position 12, which provides the two oxygen atoms necessary for the binding of Ca^{2+} [24, 25]. In addition, two helix-loop-helix motifs, a characteristic arrangement of the EF-hand domains, are predicted for Ole e 3 (Fig. 1), and its high α -helical content is also in agreement with those reported for calmodulin-related proteins [26]. These properties have also been proposed for the homologous allergen from birch pollen and are expected for those of Bermuda grass and *Brassicaceae*. Therefore, this family of pollen allergens can be considered as calmodulin-related proteins.

A comparison of the amino acid sequence of these pollen proteins with those of calbindin-D9K, S100 proteins, calgranulins, and other calmodulin-related proteins containing two Ca^{2+} -binding sites results in low values of identity/similarity, different length of the Ca^{2+} -binding site I and different length of the segment connecting the two Ca^{2+} -binding sites. When plant calmodulins are considered for the comparison, < 70% of sequence similarity and 40–50% identity are obtained. Therefore, Ole e 3 and its homologous allergens would be members of a novel family of Ca^{2+} -binding calmodulin-related proteins, for which we propose the generic name of polcalcins, because they are specifically detected in pollen tissue and bind calcium ions.

In plants, Ca^{2+} has been suggested to be involved in the signal transduction in response to external stimuli from environmental changes [27, 28], and this kind of response should be crucial in the development and metabolism of pollen. In fact, Ca^{2+} and Ca^{2+} -binding proteins seem to be involved in regulating the expression of genes related to self-incompatibility responses [29], and the growth of the pollen tube as well as its orientation are dependent on cytosolic free Ca^{2+} gradients and Ca^{2+} channels [30, 31]. By means of iontophoresis studies, it has been shown that Bet v 4 and its homologous proteins would constitute a new class of proteins that may have a role as inhibitors of cytoplasmic streaming in growing pollen tubes [15]. Further studies of their members may contribute to elucidate the regulatory influence of Ca^{2+} in the metabolism and germination of such a plant tissue.

Ole e 3 is only expressed in pollen tissue as observed for its homologues in rapeseed [12] and birch [15]. Besides, the presence of Ole e 3-like proteins in different pollens has been revealed by Western blots and Northern blots, using both Ole e 3-specific antibodies and a cDNA fragment, respectively. It is remarkable that samples such as those from birch or mugwort gave a positive response by Western-blot analysis, whereas they did not show RNA hybridisation. In this regard, ELISA inhibition assays of the binding of Ole e 3-specific antiserum to Ole e 3 by pollen extracts from mugwort, birch, ryegrass, rose, pine and cypress have previously shown values in the range 40–90% [5]. IgE-cross inhibition data were obtained for lily and timothy pollen extracts probed with Bet v 4 [15]. Accordingly, all these pollens contain Ole e 3/Bet v 4-like proteins, which can represent highly cross-reactive allergens, although they would show differences in sequence similarity or level in the pollen. Thus, the ubiquity of this family of proteins would be of great clinical importance. Olive tree belongs to the Oleaceae family, whose allergenic patterns obtained by SDS/PAGE are remarkably different from those of other pollens. However, cross-reactivity has been described between olive-tree and grass-pollen extracts of allergenic relevance [32]. Ole e 3, the prevalence of which has been shown to be higher than in 50% of the olive allergic patients in areas of high levels of olive-tree pollen [5], could be involved in a main manner in such processes. In conclusion, the proteins belonging to the proposed polcalcin family are good

candidates to be responsible of IgE cross-sensitisation, which would compel the precise diagnosis and specific immunotherapy treatments for the hypersensitive patients for which the production of recombinant Ole e 3 will be useful.

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