

Ole e 3, a Candidate for in vivo Diagnosis of Polcalcin Sensitization

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Polcalcin · Panallergen · Calcium-binding protein · Cross-reactivity · Allergen purification · Immunoaffinity chromatography

Abstract

Introduction: Polcalcins belong to the family of calcium-binding proteins. They are ubiquitous in the plant kingdom and highly conserved, which leads to these panallergens showing a high degree of inter-cross-reactivity. They are responsible for allergic polysensitization, and therefore, their diagnosis is necessary for correct selection of immunotherapy. The objectives were to develop a method to purify native polcalcin with intact allergenic properties and to validate its use for diagnosis of polcalcin sensitization. **Methods:** Ole e 3 was purified by immunoaffinity chromatography using anti-rChe a 3 polyclonal antibodies and identified by mass spectrometry. Calcium-binding assays were performed in immunoblot and ELISA assays. Diagnostic capacity of Ole e 3 was analyzed by ELISA and compared to ImmunoCAP with sera from a pollen-sensitized population. Cross-reactivity with other polcalcins was investigated by ImmunoCAP inhibition. **Results:** Immunogenicity of purified Ole e 3 was not affected by the addition of calcium. However, the presence of a cal-

cium chelator agent completely inhibited IgG binding by immunoblot and produced a 32.3% reduction in IgE binding by ELISA. Ole e 3 enabled diagnosis of polcalcin-sensitized patients, and a good correlation was revealed with ImmunoCAP. A 50% inhibition in IgE binding was obtained with 2.8 ng of Ole e 3 for rBet v 4 and 3.9 ng for rPhl p 7. **Discussion/Conclusion:** Native Ole e 3 was purified by maintaining its allergenic properties. This innovative method enables obtaining this active native allergen to be used for in vivo diagnosis of polcalcin sensitization.

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Introduction

Polcalcins are 9 kDa calcium-binding proteins identified as allergens in pollen from grasses [1, 2], weeds [3, 4], and trees [5–7]. This protein family shares common domains termed EF-hands that are able to bind and transport calcium and interact with a diversity of ligands in a calcium-dependent manner. One of these ligands is IgE, and polcalcin allergenicity is primarily associated with the

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Ca²⁺-bound form of the protein [8]. According to the number of EF-hands contained in their sequence, polcalcins can be classified into those containing 2 EF-hands, allergens Bet v 4, Che a 3, Ole e 3, Cyn d 7, or Phl p 7, the group containing 3 EF-hands, such as Bet v 3 and allergens with 4 EF-hands such as Jun o 4, Cup a 4, and Ole e 8 [9].

It has been shown that polcalcin sequences are highly conserved and show a high degree of inter-cross-reactivity [10, 11], which is responsible for polysensitization. This marked homology between polcalcins and their wide distribution throughout the plant species makes polcalcins one of the most relevant groups of panallergens. Polcalcins have been reported as relevant allergens in different plant species such as *Chenopodium album* [3] and *Olea europaea*, among others [12]. For this reason, their use in skin prick test (SPT) *in vivo* diagnosis in addition to their inclusion in component resolved diagnosis (CRD) of allergy, by *in vitro* techniques, seems to be crucial for prescription of a more precise allergen immunotherapy. Those patients with genuine sensitization or sensitization caused by panallergens that are cross-reactivity markers (especially profilins and polcalcins) are singled out. For this purpose, to date, several recombinant and natural polcalcins have been purified [3, 6, 13–15] and have been used for *in vitro* diagnosis [15] and cross-reactivity studies [9, 10].

The purification processes reported to date for native polcalcins consist of several liquid chromatography steps [3], which involved very long processes with a low protein purification yield. Recombinant proteins, as is well known, present higher purification yields [16] but also more inconveniences, from a regulatory point of view, to be used as *in vivo* diagnostic tools. Moreover, recombinant proteins do not always have the same configuration as native proteins and their allergenicity could be affected. For all these reasons, it is important to develop suitable methods for purification of native allergens to be used as diagnostic tools, especially in the case of SPT. Against this backdrop, immunoaffinity chromatography with the use of specific antibodies is an interesting alternative due to its high specificity and protein recovery capacity [17, 18].

The main aim of this study was to develop a method based on immunoaffinity chromatography for purifying native polcalcin with intact allergenic properties. This is so as to have the possibility of developing an appropriate SPT for *in vivo* diagnosis. In order to confirm its quality as a diagnostic tool, its immunogenicity in relation to the Ca²⁺ requirement, its IgE-binding capacity, and its cross-reactivity with other polcalcins were studied.

Materials and Methods

Cloning, Expression, and Purification of Recombinant Che a 3

C. album RNA was obtained from 20 mg of freshly harvested pollen (Iberpolen, Jaén, Spain) using the FastPrep-24 and Fast-RNA Pro kit (MP Biomedicals, Irvine, CA, USA) according to manufacturer's instructions. cDNA was synthesized and amplified with Che a 3 specific primers FW-Pol Che2 5' **CGGATCCAATG-GCTGCTGAGG** 3' and RV-Pol Che1 5' **GGAAGCTTCTAGAA-GATCTTGGAAACATCT**3' (Bam HI and Hind III restriction sites appear in bold and start and stop codons in italics).

The fragment obtained was cloned in pQE-31 using the Bam HI and Hind III sites. The construction pQE-Che a 3 was transformed into *Escherichia coli* M15 strain. Recombinant Che a 3 was induced by the addition of 0.4 mM IPTG and incubation at 30°C for 5 h. After induction, the bacterial culture was recovered and homogenized in phosphate buffer 20 mM pH 7.4, NaCl 0.5 M, imidazol 20 mM, and protease inhibitor cocktail (1 mL/20 g of bacterial pellet; Sigma-Aldrich, St. Louis, MO, USA). The solution was sonicated at 55% of amplitude for 8 cycles of 15 s. After centrifugation for 20 min at 10,000 rpm and 4°C, the supernatant was recovered and passed through a His-Trap column in an ÄKTA explorer instrument (GE Healthcare, Uppsala, Sweden) for rChe a 3 purification. Protein was eluted with a linear gradient from 0 to 100% of phosphate buffer 20 mM pH 7.4, NaCl 0.5 M, and imidazol 500 mM. Fractions were analyzed by SDS-PAGE, and those containing rChe a 3 were pooled and finally separated from other high molecular weight proteins by an Amicon centrifugal device of 30 kDa (Merck Millipore, Darmstadt, Germany). The protein obtained was analyzed by SDS-PAGE under reducing conditions and sequenced by liquid chromatography/mass spectrometry-mass spectrometry (LC/MS-MS) at the CNB-CSIC Proteomics Service to confirm its identity.

Production of anti-Che a 3 Polyclonal Antibodies

Two New Zealand White rabbits were immunized at Isoquimen (Barcelona, Spain) with 330 µg of rChe a 3 in complete Freund's adjuvant and with 165 µg of rChe a 3 in incomplete Freund's adjuvant as boosters on days 22, 43, and 64. Sera were obtained after 78 days. All procedures were in accordance with EU and local legislation and were approved by the Ethics and Animal Experimentation Committee.

Purification of anti-Che a 3 IgG Antibodies

Rabbit sera were filtered, diluted in phosphate buffer 40 mM pH 7, and purified with protein A HiTrap column in an ÄKTA explorer system (GE Healthcare). IgG antibodies were eluted with a linear gradient from 0 to 100% of glycine-HCl buffer 100 mM pH 2.7. Tris-HCl 1 M pH 9 buffer was added to fractions for neutralization. Purified IgG was quantified, titrated, and stored at –20°C.

Immunoaffinity Chromatography

Column Preparation

Purified IgG-polyclonal antibodies anti-rChe a 3 were dialyzed against a coupling buffer (NaHCO₃ 0.1 M; NaCl 0.5 M) and bound overnight at 4°C to a CNBr-Sepharose 4B resin (GE Healthcare) previously activated in HCl 1 mM, according to manufacturer's instructions. After incubation for 2 h with Tris-HCl 0.1 M pH 8.0, the resin was packed in a XK 16/20 column (GE Healthcare). Fi-

nally, the resin was washed consecutively with regeneration buffer 1 (CH₃COOH 0.1 M pH 4.0; 0.5 M NaCl) and regeneration buffer 2 (Tris-HCl 0.1 M pH 8.0; 0.5 M NaCl) and stored in 20% ethanol at 4°C until use.

Native Polcalcins Purification

100 mg of *C. album* or *O. europaea* pollen lyophilized extracts were dissolved in phosphate buffer 20 mM pH 7.0 and filtered by a 0.45 µm membrane. Immunoaffinity chromatography was performed in an ÄKTA explorer system (GE Healthcare) using the previously packed column. Proteins were eluted with a pH change using Glycine-HCl 100 mM pH 2.7 as elution buffer. Fractions were analyzed by SDS-PAGE and those containing polcalcin were recovered, mixed, and concentrated with Amicon 3k devices (Merck Millipore).

Polcalcin Characterization

Purified proteins were loaded in SDS-PAGE with 2.67% C, 15% T acrylamide under reducing conditions and stained with Oriole (BioRad, Hercules, CA, USA). Protein identity was verified by trypsin digestion and sequencing by LC/MS-MS.

Serum Samples from Allergic Patients

A total of 92 serum samples from polysensitized allergic patients, who previously gave their written consent, were recruited at Hospital Universitario de Guadalajara (Spain). This study was approved and authorized by the scientific Ethics Committee of the Hospital Universitario de Guadalajara (No. 01/2009) [19]. Equal volumes of serum samples from 6 polcalcin positive patients were used to prepare a pool for calcium-binding assays (sIgE values to rPhl p 7 ranging from 2.94 to 56.1 kUA/L).

Calcium-Binding Assays

Addition or depletion of Ca²⁺ was assayed with Ole e 3 in immunoblot and ELISA assays by incubating the protein with 0.1 mM CaCl₂ or 1 mM EGTA pH 7.5 (Ca²⁺ chelator agent) together with the polyclonal antibody (IgG) in immunoblot or the pool of sera from polcalcin positive patients (IgE) in ELISA assays. In short, purified Ole e 3 SDS-PAGE was transferred onto a Trans-Blot® Turbo™ Transfer Pack (BioRad), blocked with 5% skimmed milk in PBS-T (PBS with 0.01% Tween-20) for 1 h and incubated overnight with polyclonal antibodies anti-rChe a 3 (1/40,000 diluted), with or without Ca²⁺ or EGTA. Subsequently, the membrane was washed, incubated with anti-IgG-PO, and developed with ImmunoStar™ Western Kit (BioRad).

For the ELISA assays, microtiter plates (Immulon 4HBX, Thermo Fisher Scientific) were coated with Ole e 3 (5 µg/mL), incubated overnight, and blocked with 1% BSA in PBS-T. The plate was then incubated for 2 h with the pool of sera at a dilution 1/2 or with the sera plus Ca²⁺ or EGTA, washed, and incubated for an additional 2 h with peroxidase-conjugated monoclonal anti-human IgE (Ingenasa, Madrid, Spain). After washing, the reaction was developed, stopped, and read at 450 nm.

IgE-Binding Studies

ELISA assay was performed to identify Ole e 3 sensitized patients in the 92 sera included in this study. The assay was performed as previously reported, using each individual sera at a dilution 1/2 (1:1 diluted in 0.01 M PBS). Positive patients were considered those with optical density (OD) higher than 0.126 (cutoff

established as 3 × mean OD of negative control). In addition, polcalcin-specific IgE values from the grass *Phleum pratense* (rPhl p 7) were determined by the ImmunoCAP 100E system (Thermo Fisher, Uppsala, Sweden) in 45 of the serum samples analyzed by ELISA, including positive and negative samples.

Cross-Reactivity

ImmunoCAP inhibition assays were performed to investigate IgE cross-reactivity between purified Ole e 3 and the polcalcins rBet v 4 and rPhl p 7 (Thermo Fisher). Different aliquots of the pool of sera (diluted 1/2) were previously incubated with Ole e 3 amounts ranging from 0.6 to 312.5 ng/mL (0.02–12.5 ng). The same dilution of the pool of sera in 0.01 M PBS without Ole e 3 was used as a control. After 2 h of incubation, specific IgE (kUA/L) binding to rBet v 4 or rPhl p 7 was determined in all samples.

Statistical Analysis

Spearman correlation test was used to identify linear relationships between the techniques used for identification of polcalcin positive patients. Differences were considered statistically significant if $p < 0.05$. Descriptive statistical analyses (mean and SD) were used for the analysis of numerical variables (age). Fisher's exact test was used to compare groups of individuals (those positive to Ole e 3 vs. those negative to Ole e 3). Statistical analysis was performed using GraphPad Prism 7.02 software (GraphPad Software, San Diego, CA, USA).

Results

Cloning, Expression, and Purification of Recombinant Che a 3

The ORF corresponding to Che a 3 contained an 86 amino-acid sequence, corresponding to the sequence that appears in UniProtKB (Q84V36) with the addition of 13 amino acids in the N-terminus including the 6-Histidine tag. Based on the ProtParam tool (<https://web.expasy.org/protparam/>), the protein had a molecular mass of 11.1 kDa and a theoretical pI of 5.2.

The rChe a 3 yield obtained after expression and purification was approximately 10 mg/L of bacterial culture. Protein purity and identity were confirmed by SDS-PAGE (Fig. 1a) and LC/MS-MS. A sequence coverage of 90% was obtained (Fig. 1b).

Production and Purification of anti-Che a 3 Polyclonal Antibodies

Sera from the two immunized rabbits had similar titres of specific IgG whereas both pre-immune sera were negative. After IgG purification, both sera maintained a high titre for purified rChe a 3 as well as for the whole *C. album* extract (data not shown).

Fig. 1. Characterization of purified rChe a 3. **a** SDS-PAGE of rChe a 3 after the affinity chromatography step (line 1) and after centrifugation with filter devices (line 2). **b** LC/MS-MS results. Peptides obtained covering 90% of the sequence are marked in bold. LC/MS-MS, liquid chromatography/mass spectrometry-mass spectrometry.

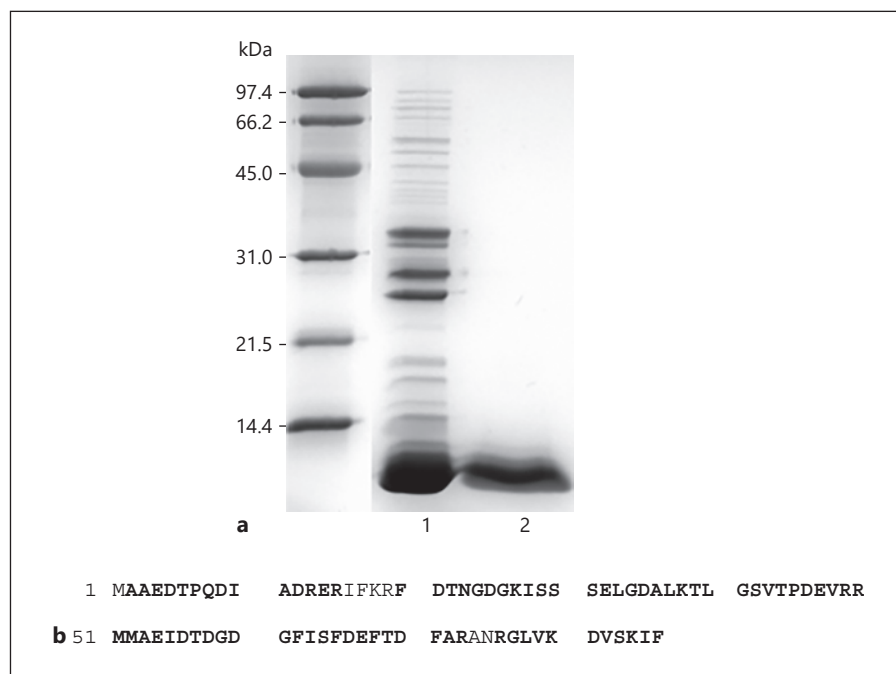
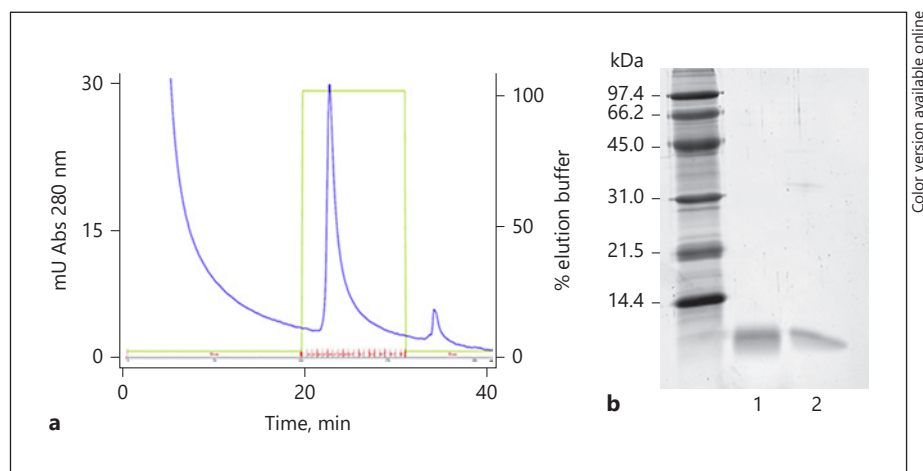


Fig. 2. Purification and characterization of native polcalcins. **a** Chromatographic profile characteristic of immunoaffinity purification of native polcalcin showing the peak corresponding to the purified protein (blue line) and the percentage of elution buffer (green line). **b** SDS-PAGE of purified Ole e 3, line 1 and Che a 3, line 2.



Immunoaffinity Chromatography and Polcalcin Characterization

C. album and *O. europaea* polcalcins were purified by affinity chromatography. Both proteins were recognized by anti-Che a 3 polyclonal antibody (online suppl. Fig. 1; see www.karger.com/doi/10.1159/000512303 for all online suppl. material). A single chromatographic peak as shown in Figure 2a containing a band with the predicted molecular weight of approximately 9 kDa was obtained in both processes (Fig. 2b). Che a 3 and Ole e 3 were identified by LC/MS-MS with sequence coverage of 90 and 68%, respectively (Fig. 3a). The sequences obtained

corresponded to the UniprotKB ID Q84V36 (Che a 3) (online suppl. Fig. 2) and O81092 (Ole e 3) (online suppl. Fig. 3) that present 81% identity determined by Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). Purified Ole e 3 was selected for subsequent studies. Average yield of Ole e 3 purification was 0.25% from the lyophilized extract.

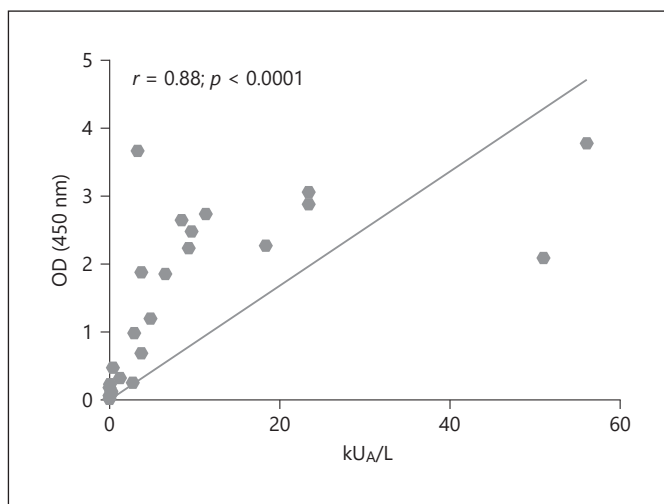
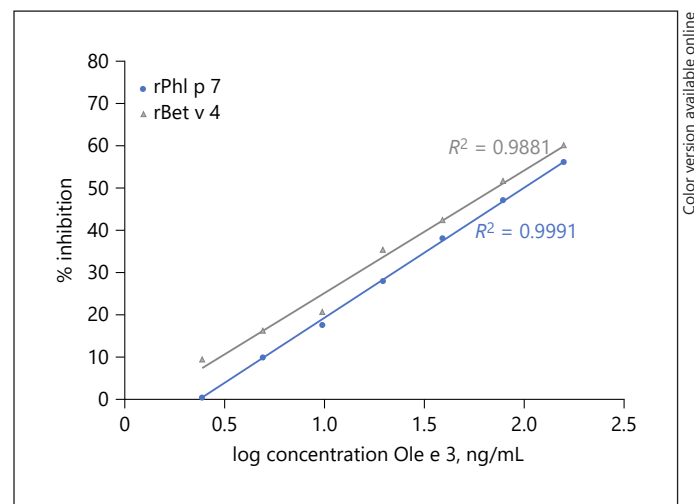
Calcium-Binding Assays

There were no differences in polcalcin binding to IgG or IgE antibodies depending on whether or not Ca^{2+} was present (shown in Fig. 4). However, addition of EGTA to

Table 1. Description of the study population

	Total	Pool of sera	Ole e 3 positive	Ole e 3 negative	<i>p</i> value
<i>N</i>	92	6	24 (26.1%)	68 (73.9%)	
Age, years±SD	29.1±12.7	30.5±8.5	25.6±14.0	31.1±11.6	ns
Female, <i>n</i> (%)	56 (60.9)	3 (50)	14 (58.3)	42 (61.8)	ns
<i>Respiratory symptoms, n (%)</i>					
Rhinitis	92 (100)	6 (100)	24 (100)	68 (100)	ns
Conjunctivitis	72 (78.3)	6 (100)	17 (70.8)	55 (80.9)	ns
Asthma	51 (55.4)	5 (83.3)	17 (70.8)	34 (50.0)	ns
<i>Sensitization to pollen, n (%)</i>					
Grasses	62 (67.4)	6 (100)	24 (100)	38 (55.9)	<0.0001
Chenopodium	45 (48.9)	5 (83.3)	15 (62.5)	30 (44.1)	ns
Olea	81 (88.0)	6 (100)	23 (95.8)	59 (86.8)	ns
Cupressus	40 (43.5)	5 (83.3)	14 (58.3)	26 (38.2)	ns

Percentages calculated from the “*n*” in each group. *p* value calculated comparing Ole e 3 positive and negative patients (Fisher’s exact test). ns, nonsignificant.

**Fig. 5.** Correlation between sIgE levels obtained by ELISA (OD) with purified Ole e 3 or ImmunoCAP (kUA/L) with rPhl p 7. OD, optical density.**Fig. 6.** Inhibition of IgE binding to rPhl p 7 (blue circle) and rBet v 4 (gray triangle) with purified Ole e 3 by ImmunoCAP.

Cross-Reactivity

A 50% inhibition of IgE binding to rBet v 4 is obtained with 2.8 ng of Ole e 3 (70.4 ng/mL) whereas 3.9 ng (97.2 ng/mL) are necessary for a 50% inhibition of IgE to rPhl p 7 as shown in Figure 6. The maximum rate of inhibition attained was >60% for both polcalcins (68.6% for rBet v 4 and 61.6% for rPhl p 7).

Discussion

Profilins [20] and polcalcins are important respiratory panallergens. Despite their ubiquity in the plant kingdom, both panallergens are not recognized as major allergens and their clinical relevance remains poorly understood. However, their role as a marker of polysensitization and their responsibility in cross-reactivity among pollens is clearly stated [21]. These characteristics high-

light the importance of diagnosing polcalcin sensitization in plant allergic patients to support selection of the most suitable allergen immunotherapy extracts for each patient [22]. It has been estimated that 10% of pollen allergic individuals are sensitized to polcalcins, although this percentage is highly variable depending on the specific source of pollen causing the sensitization, geographical factors, etc. [11]. However, and in spite of numerous studies that report the role of polcalcins as relevant panallergens, there are no currently commercially available SPT of purified polcalcin for *in vivo* diagnosis.

Polcalcins are generally present in low amounts in allergenic extracts. Therefore, their purification with a high degree of purity, quality, and sufficient amount to manufacture SPT by keeping their allergenic properties, and structure intact is a challenge. Under this scenario, the aim of this study was to develop a new purification method to obtain native polcalcin whilst keeping its allergenic properties intact, including IgE-binding capacity and cross-reactivity with other proteins from the same family.

Polcalcins from *O. europaea* (Ole e 3) and *C. album* (Che a 3) pollen have been reported as relevant allergens in sensitized patients [3, 12]. Based on this, both allergens were selected as optimal options to develop SPT; they were validated as potential proteins for polcalcin sensitization detection.

Purification of native allergens is a relevant and useful tool, not only for using the allergens for *in vivo* or *in vitro* diagnosis but also for development of allergen quantification kits or to be used as standards for allergen extracts characterization. Contrary to recombinant allergens, native allergens purified from natural sources usually maintain their immunological and allergenic properties and are considered a better option to be used as diagnostic tools. However, obtaining them requires more complicated and expensive methods and with lower yields. In this regard, immunoaffinity chromatography provides a highly specific way for purification of proteins by means of interaction with their specific antibodies. For this purpose, anti-Che a 3 antibodies were used for purification of polcalcin in only one step, which maximized the amount of protein obtained whereas protein impurities are avoided in the sample. After purification of Che a 3 and Ole e 3, the latter was selected because a higher yield was obtained. The yield obtained for Ole e 3 purification by this methodology (0.25%) represents an increase of more than 40 times compared to previously published data (0.006% [3]).

Regarding immunological and allergenic activity, IgE binding of polcalcins depends on the native protein conformation and required protein-bound Ca^{2+} [8]. We dem-

onstrated that Ole e 3 purified by affinity chromatography did not require addition of Ca^{2+} to bind to the immunoglobulins present in polyclonal sera or in serum samples from sensitized patients. However, depletion of Ca^{2+} with a calcium chelator agent completely inhibited IgG binding and reduced IgE binding by 32%, which confirms the role of Ca^{2+} in the protein's immunological properties. Similar results of calcium-dependent IgE recognition have been obtained in other studies performed with allergens from the calcium-binding protein family such as fish parvalbumin [23], Bet v 4 [5], and Phl p 7 [24].

The utility of purified Ole e 3 for identifying polcalcin-sensitized individuals was demonstrated by an ELISA test, in which 24 out of 92 serum samples from pollen allergic patients were identified as positive. This positivity was confirmed by ImmunoCAP in 19 cases and revealed a statistically significant correlation ($p < 0.0001$) between the values obtained by both techniques. Differences in the number of positive patients identified by the 2 techniques could be explained by a different sensitivity of the techniques or the primary sensitizer considering that the patient population presented multiple pollen allergies. In addition, the 5 ELISA positive and ImmunoCAP negative patients presented OD values close to the positivity threshold.

Cross-reactivity between different polcalcins has been previously reported using whole pollen extracts [10] or recombinant polcalcins [3, 6, 25]. In our work, we demonstrated cross-reactivity between the purified native Ole e 3 and the 2 recombinant polcalcins available for ImmunoCAP (rPhl p 7 and rBet v 4). The amount of Ole e 3 necessary to obtain a 50% inhibition of the sera pool was similar for both proteins. Higher inhibition was obtained with rBet v 4 which could be accounted for by the higher number of Phl p 7 IgE epitopes [10] and because patients included in the pool of sera were selected by their sensitization to rPhl p 7.

CRD with purified panallergens has become a relevant and potent tool for diagnosis of allergy, especially in polysensitized patients. Specifically, SPT prepared from these purified allergens is an efficient and economic tool for better characterization of patients and immunotherapy prescription. The IgE-binding capacity of purified Ole e 3 demonstrated by ELISA and ImmunoCAP experiments confirmed this purified allergen's allergenicity and diagnostic capacity. These results, therefore, open up the possibility of using Ole e 3 for diagnosis by SPT, which is an important unmet need in CRD with panallergens. The main challenge we have now is to perform a clinical study for determining the product's specificity and sensitivity. These studies also open up the possibility of treating pa-

tients with panallergens or even, to be used as a natural source for enriching allergen extracts with specific purified allergens.

In summary, we have developed a method for purification of native polcalcins by a single step of affinity chromatography that could be valid for purification of polcalcins from different allergenic sources due to the high percentage of identity between the amino acid sequences and because they present a high cross-reactivity. Therefore, this is a very promising method for the purification of allergens with high cross-reactivity. The purified Ole e 3 obtained by this method contains the Ca²⁺ necessary to maintain its allergenicity and has been demonstrated to be cross-reactive with other polcalcins. Therefore, this new method is an ideal tool to obtain immunologically active native polcalcins to be used in SPT for *in vivo* diagnosis of allergic patients.

Statement of Ethics

All patients signed the informed consent form, and this study was approved by the Scientific Ethics Committee of the Hospital Universitario de Guadalajara (study No. 01/2009).

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Conflict of Interest Statement

R.M., A.G.R., M.A.L., and J.C. are employees of Laboratorios LETI S.L.U. J.M.B. has no conflicts of interest to declare.

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Author Contributions

R.M. contributed to the design of the work, analysis and interpretation of data, drafting the work, and final approval and agreed to be accountable for all aspects of the work. A.G.R. contributed to the acquisition and analysis of data, revising the work critically, and final approval and agreed to be accountable for all aspects of the work. J.M.B. contributed to the acquisition of data, revising the manuscript, and final approval and agreed to be accountable for all aspects of the work. J.C. contributed to the design of the work, revising the manuscript critically, and final approval and agreed to be accountable for all aspects of the work. M.A.L.M. contributed to the design of the work and interpretation of the data, drafting the work, and final approval and agreed to be accountable for all aspects of the work.

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Supplementary material

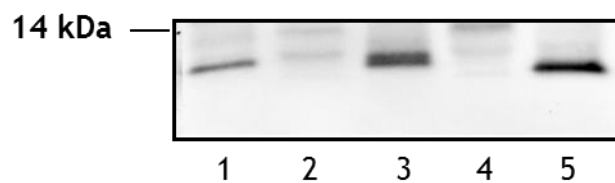


Figure 1S: Immunoblot of 1- *Chenopodium album*, 2- *Cynodon dactylon*, 3- *Olea europea*, 4- *Parietaria judaica* and 5- *Phleum pratense* extracts incubated with polyclonal antibody anti-Che a 3 at a dilution 1:10000.

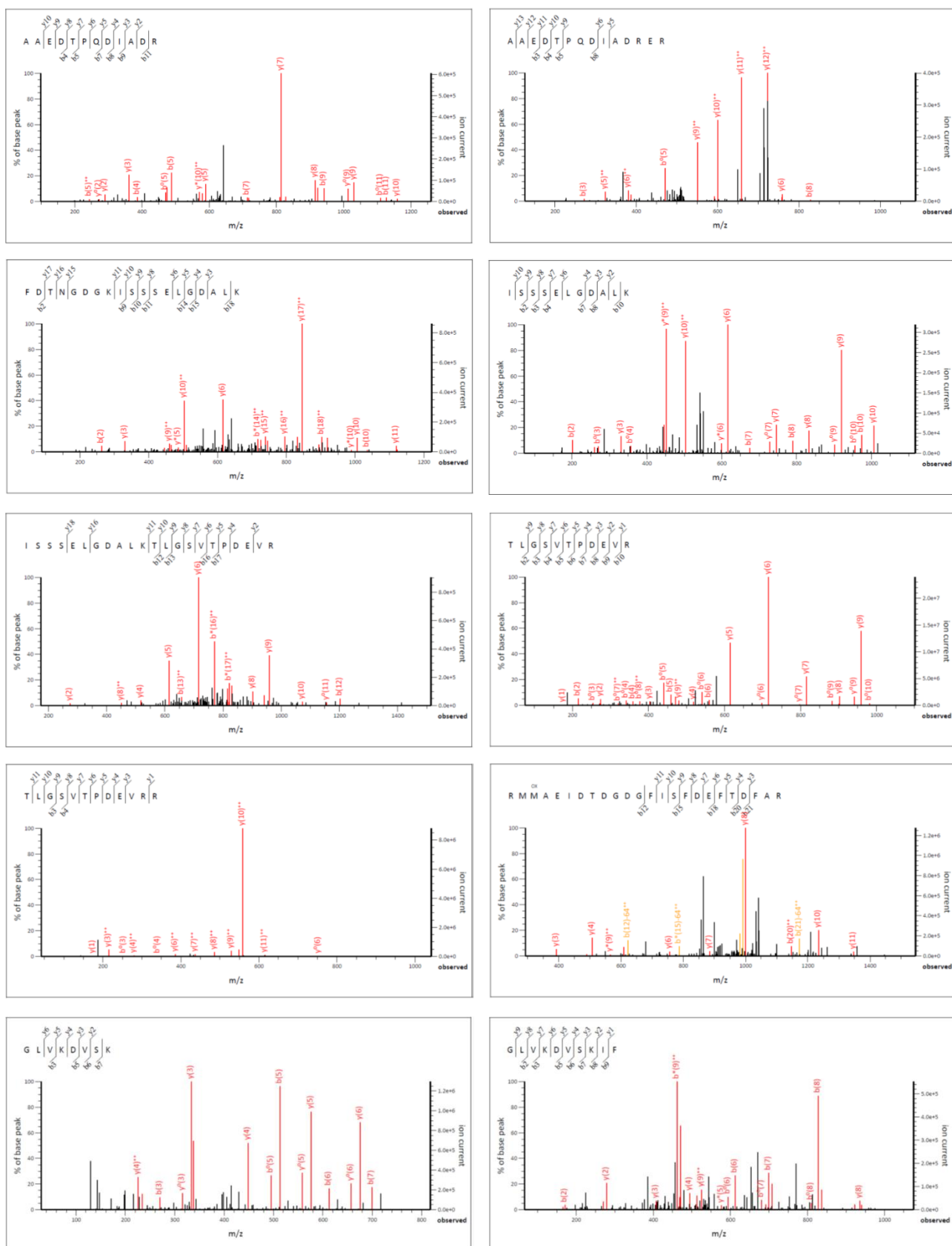


Figure 2S: Peptides of Che a 3 obtained after trypsin digestion and analysis by LC/MS-MS. All the peptides were found in Q84V36 in UKBsp_all, Polcalcine Che a 3 OS=*Chenopodium album*.

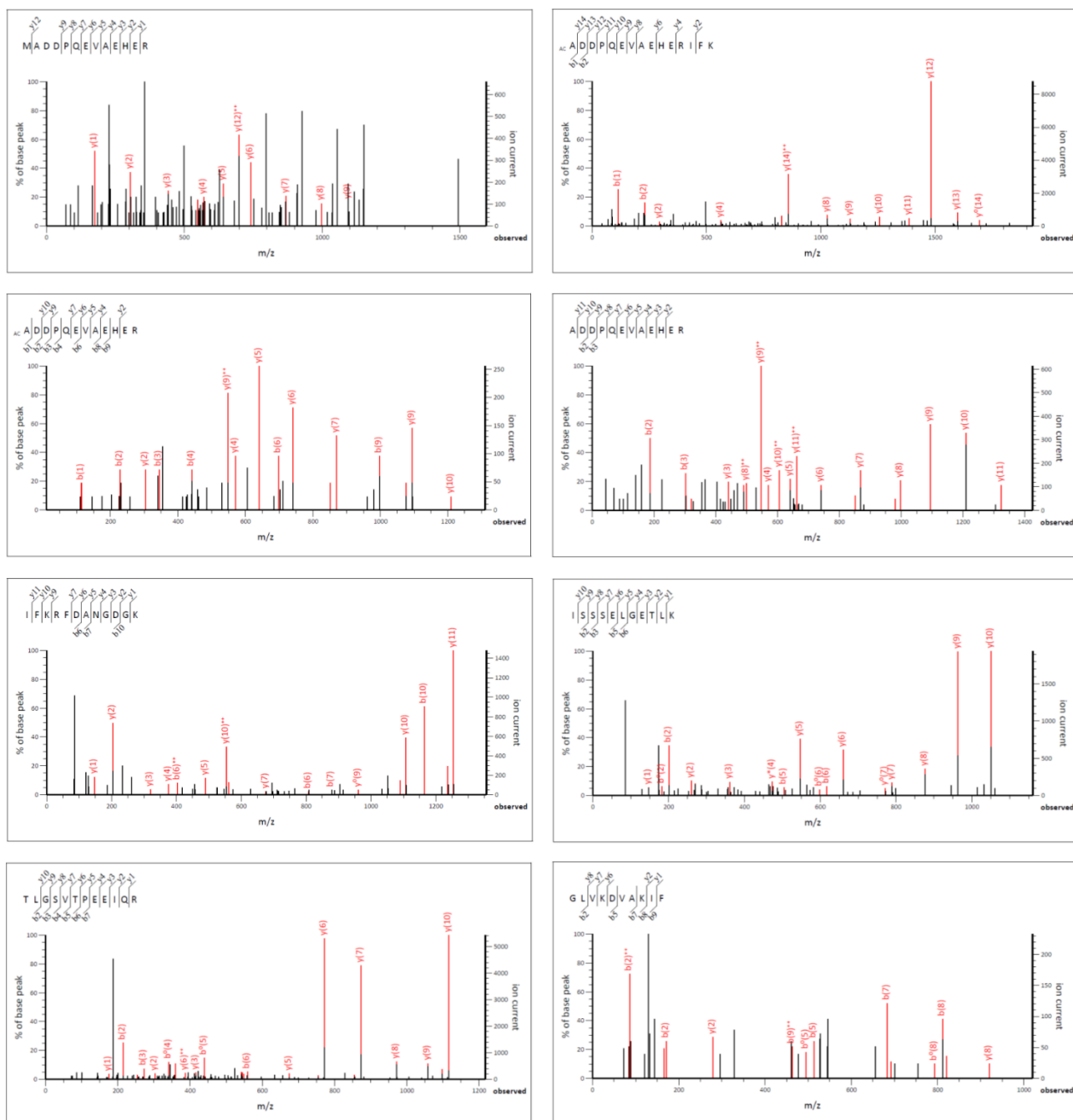


Figure 3S: Peptides of Ole e 3 obtained after trypsin digestion and analysis by LC/MS-MS. All the peptides were found in O81092 in UKBsp_all, Polcalcin Ole e 3 OS=*Olea europaea*.

Supplementary Material Table 1S. specific IgE values to Ole e 3 (by ELISA) and rPhl p 7 (by ImmunoCAP) of patient population selected for correlation study

<i>Serum ID</i>	<i>Ole e 3 O.D. (ELISA)</i>	<i>rPhl p 7 kUA/L (ImmunoCAP)</i>
1	0.88	2.94
2	3.65	56.1
3	1.52	3.76
4	3.35	3.31
5	1.71	51.0
6	1.54	6.55
7	2.05	9.69
8	0.10	0.31
9	0.03	0.00
10	0.16	0.01
11	0.05	0.01
12	0.53	3.83
13	1.78	9.35
14	1.87	18.4
15	0.19	2.74
16	0.04	0.16
17	0.94	4.81
18	2.85	23.4
19	2.79	23.4
20	0.12	0.02
21	0.00	0.00
22	0.04	0.17
23	0.00	0.00
24	0.12	0.02
25	0.89	4.92
26	0.01	0.01
27	0.04	0.02
28	0.24	1.25
29	0.16	0.09
30	0.07	0.03
31	0.19	0.12
32	0.07	0.03
33	0.07	0.03
34	0.07	0.01
35	0.07	0.11
36	0.05	0.01
37	0.05	0.08
38	0.01	0.00
39	0.07	0.03
40	0.06	0.01
41	0.35	0.46
42	0.17	0.03
43	0.16	0.02
44	2.18	8.49
45	2.23	11.3