

Identification of a polygalacturonase as a major allergen (Pla a 2) from *Platanus acerifolia* pollen

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Background: Planetree pollen allergy is a clinical disorder affecting human populations in cities of the United States and Western Europe, but little is known about its relevant allergens. **Objective:** We sought to purify, characterize, and clone the 43-kd allergen from *Platanus acerifolia*.

Methods: *P. acerifolia* pollen extract was fractionated by using ion-exchange and gel-permeation chromatography. Analyses were carried out by using ELISA, SDS-PAGE, isoelectrofocusing, and immunoblotting. Partial amino acid sequence was obtained by means of Edman sequencing of cyanogen bromide–digested peptides. Specific cDNA was cloned by using reverse transcription, followed by PCR, with amino acid sequences from peptides of the allergen.

Results: The allergen isolated from *P. acerifolia* pollen, Pla a 2, is a glycoprotein with an observed molecular mass of 43 kd and an isoelectric point value of 9.3. It is involved in the allergic responses of 84% of patients with planetree-induced pollinosis and represented 52% of the total IgE-binding capacity of the *P. acerifolia* extract. Pla a 2 displays polygalacturonase (PG) activity, being the first PG with functional enzyme activity from an angiosperm plant pollen described as an allergen. The cDNA allergen sequence codified for a 372-residue protein with 56% and 42% sequence identity to PGs from pollen and fruits, respectively. Western blot analysis showed that Pla a 2 is present in pollen and stems and has IgG cross-reactivity with a PG from tomato and pectate lyases from *Cupressaceae* pollen.

Conclusion: Pla a 2, a major allergen of *P. acerifolia* pollen with PG activity has been purified, characterized, and cloned. (J Allergy Clin Immunol 2004;113:1185-91.)

Key words: London planetree, major allergen, *Platanus acerifolia*, polygalacturonase, three-dimensional structure, molecular modeling

Type I allergic disorders, such as rhinoconjunctivitis, eczema, and asthma, are a global problem that afflict up to 40% of the population in industrialized countries.¹ Identification, isolation, and characterization of allergens

Abbreviations used

CNBr: Cyanogen bromide
IEF: Isoelectrofocusing
PG: Polygalacturonase
PVDF: Polyvinylidene difluoride

is a necessary task to improve the diagnosis and treatment of this increasing clinical disorder and can help to explain relationships among biologic function, protein structure, and allergenic activity.² Unfortunately, a relatively small number of allergens have been biochemically characterized among pollen allergens.

London planetree (*Platanus acerifolia*) is an urban tree of choice in North America, Europe, and Australia because of its resistance to diseases and especially to the air pollution found in the urban environment. High concentrations of its pollen are detected during the flowering season in several European countries,³ reaching up to 14% of total pollen in Madrid (Spain).⁴ The other important planetree is the American sycamore (*Platanus occidentalis*), which is common in the eastern deciduous forests of the United States. Two allergens have been described as important components of *P. acerifolia* extracts, Pla a 1 and a 43-kd protein,^{5,6} with the former recently cloned and identified as an invertase inhibitor.⁷

Polygalacturonases (PGs) catalyze the degradation of highly polymeric galacturonate, a major component of pectin in plant cell walls, into individual galacturonic acid residues. PG belongs to family 28 of glycosyl hydrolases and has been identified in ripening fruit, abscission, dehiscence, pollen maturation, and rapidly expanding tissues.⁸

In the present study we describe the purification and characterization of a major allergen from *P. acerifolia* pollen, named Pla a 2 according to the standard nomenclature of allergens.⁹ The biochemical properties, allergenic significance, and cDNA sequence of this allergen are presented.

METHODS

Purification of the 43-kd allergen and N-terminal amino acid analysis

Crude extracts of *P. acerifolia* pollen (IberPolen, Málaga, Spain) were obtained by means of PBS extraction under standard conditions.⁵ *P. acerifolia* pollen extract was adjusted to pH 7.0 with 20 mmol/L phosphate buffer, chromatographed in a 1-mL HiTrap SP

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Supported by Grants FIT-090000-2003-61 from the Plan Nacional de I+D (Programa PROFIT, Ministerio de Ciencia y Tecnología, Spain) and TEI-0163-2002 from the Programa INTEK (Departamento de Industria, Agricultura y Pesca, Gobierno Vasco).

Received for publication November 28, 2003; revised February 4, 2004; accepted for publication February 18, 2004.

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0091-6749/\$30.00

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doi:10.1016/j.jaci.2004.02.031

cationic column (Amersham Biosciences, Uppsala, Sweden), and equilibrated with the same buffer on an ÄKTA-Prime System (Amersham) by using a step gradient of 0.2, 0.5, and 1.0 mol/L NaCl. The IgE-binding capacity of the fractions was analyzed by means of SDS-PAGE immunoblotting incubated with a pool of 43-kd band-reactive sera from patients with *P. acerifolia* allergy. IgE-binding proteins were concentrated and applied to a HiLoad 16/60 Superdex 200 gel filtration column (Amersham) equilibrated with PBS. Each of the peaks were pooled separately and analyzed by means of SDS-PAGE immunoblotting. Fractions corresponding to 35 to 45 kd and reacting to the positive sera were pooled, dialyzed, concentrated with Ultrafree devices (Amicon, Billerica, Mass), and kept at -20°C . Protein content was determined by using the Bio-Rad Protein Assay (Bio-Rad, Hercules, Calif).

PG assay

Pla 2 PG activity was measured with 5 μg of protein in 50 mmol/L sodium acetate buffer, pH 5.0, and 0.1 to 0.6 mg/mL poly-D-galacturonic acid methyl ester (pectin from citrus peel; Fluka, Buchs, Switzerland) as a substrate for 15 minutes at 37°C . Release of reducing sugars was detected with the neocuproine reagent (Sigma, St Louis, Mo) and measured at 450 nm with galacturonic acid as a standard.¹⁰

Cyanogen bromide cleavage of Pla 2 and peptide sequencing

For cyanogen bromide (CNBr) cleavage, 5 μL of 5 mol/L CNBr was added to 5 μg of Pla 2 dissolved in 95 μL of 70% formic acid and incubated overnight in the dark. The reaction was diluted with 0.9 mL of water and freeze-dried. CNBr-cleaved peptides were fractionated by means of reverse-phase HPLC on a Vydac C-18 column (Grace-Vydac, Hesperia, Calif) with an acetonitrile gradient (0% to 40%) in 0.1% trifluoroacetic acid. N-terminal amino acid sequences of peptides were determined by using Edman degradation with a 473A Sequencer (Applied Biosystems, Weiterstadt, Germany).

Human sera and experimental rabbit antiserum

Clinical data of patients used in this study are shown in Table I. Patients showed immediate hypersensitivity to *Platanus* species on the basis of positive skin prick test responses (Bial-Aristegui, Bilbao, Spain); a clinical history of seasonal or perennial rhinitis, asthma, or both; and specific IgE levels to London planetree pollen of greater than class 2. Specific IgE levels were quantified by means of an enzyme-linked allergosorbent test (Hycor Biomedical, Kassel, Germany), as described by the manufacturer, by using *P. acerifolia* extract coupled to paper disks.¹¹

Polyclonal antibodies were obtained in New Zealand rabbits by immunizing with 5 boosts of 200 μg of natural Pla 2 emulsified in CFA (Difco, Detroit, Mich) every 2 weeks. Sera collected 10 days after the last injection were tested by ELISA and stored at -80°C .

Electrophoresis and carbohydrate detection

SDS-PAGE was performed in 12.5% polyacrylamide gels and stained with Coomassie Blue R250 or electrophoretically transferred onto polyvinylidene difluoride (PVDF).¹² Blotted proteins were subjected to periodate oxidation (deglycosylation) after absorption on membranes by means of incubation with 10 mmol/L sodium metaperiodate in 50 mmol/L sodium acetate (pH 4.5) for 1 hour at room temperature in the dark. After intensive washes with water, membranes were blocked and incubated overnight at 4°C with rabbit antiserum against Pla 2 (diluted 1:1,000,000). Bound IgE was detected as previously described⁵ by using ECL Plus Western Blotting Detection Reagents (Amersham). Electrophoretic patterns were evaluated with a GS-710 Image Analyser (Bio-Rad). Carbo-

hydrate detection of blotted samples was performed by using the DIG Glycan Detection Kit (Roche, Mannheim, Germany), according to the manufacturer's recommendations.

Immunoblotting and specific IgE quantitation

Proteins transferred onto PVDF membranes were immunostained as described previously.⁵ Membranes were incubated overnight at 4°C with a pool of reactive sera (diluted 1:4). For inhibition experiments, different amounts of Pla 2 or BSA were preincubated overnight at 4°C with pooled sera (diluted 1:4) before exposure to membranes. Bound IgE was detected as previously described⁵ by using ECL Plus (Amersham).

ELISA was carried out in microtiter plates (Greiner, Frickenhausen, Germany), which were coated with 25 $\mu\text{g/mL}$ *P. acerifolia* extract or 2 $\mu\text{g/mL}$ Pla 2 and incubated with individual sera, as previously described.⁷ For ELISA inhibition assays, a pool of reactive sera (diluted 1:4) preincubated overnight at 4°C with each inhibitor or BSA was added to the plates and developed as previously described.⁷

Isoelectrofocusing

Isoelectrofocusing (IEF) was performed on Isogel pH 3-10 plates (FMC, Rockland, Minn), according to the manufacturer's recommendations. Separated proteins were capillary blotted¹³ onto PVDF, blocked for 1 hour at room temperature with 0.1% Tween-20 in Tris-buffered saline, and incubated as described above.

PCR-based cloning of Pla 2 cDNA and nucleotide sequence determination

Poly(A⁺)-enriched RNA was isolated from *P. acerifolia* pollen by using the MicroRNA Purification Kit (Amersham). First-strand cDNA (3' and 5') were synthesized from 1 μg of poly(A⁺)-enriched RNA by using the SMART RACE cDNA Amplification Kit (Clontech, Palo Alto, Calif) according to the manufacturer's recommendations.

An internal peptide sequence and a highly conserved amino acid region of different PGs were used to design degenerate oligonucleotides for cloning: 43-19R, SWIGTIGCHGGHCHCCIGC-HCC, and PECTF, TGYGGDCCDGGDCAYGGDAT, respectively (H represents A/T/C, Y represents C/T, D represents G/A/T, W represents A/T, S represents C/G, and I represents inosine). cDNA (3') was subjected to PCR amplification with primers PECTF and 43-19R, and a fragment of 450 bp was obtained, purified, and cloned into the pGEM vector (Promega, Madison, Wis). On the basis of the sequences obtained, 2 primers (43SECF, TACGTGGGATAA-CAGTGAAGGGCTGC, and 43SECR, GTGCAGCCCTTCACT-GTTATCCCACG, respectively) were synthesized for PCR amplification of the Pla 2 ends by using 5'- and 3'-cDNA, respectively. The partial sequences allowed the design of primers 43EXF (AGTGGTAGTGTTCATATGTG) and 43EXR (TTAGGCACAT-TTAATAGCTGG) to determine the full-length of the nucleotide-coding sequence of Pla 2 cDNA.

Homology modeling

Homology searches and multiple amino acid sequence alignments were performed by using the BLAST and CLUSTAL programs, respectively. Sequence-based secondary structure was predicted with the Protein Structure Prediction server PSIPRED 2.4.¹⁴ Homology modeling of the 3-dimensional structure of Pla 2 was generated by using the SWISS-MODEL server (<http://www.expasy.ch/swissmod/SWISS-MODEL.html>) on the basis of the PG from *Erwinia carotovora* (PDB accession no. 1BHE).¹⁵

TABLE I. Clinical data of patients allergic to *P acerifolia*

Patient no.	Sex	Age (y)	Symptoms	<i>P acerifolia</i> extract		Specific IgE to Pla a 2	Other sensitizations
				Specific IgE (kU/L)	SPT (mm ²)		
1	F	41	RC, A	66.50	62	+	None
2	M	50	R, A	1.67	73	+	None
3	F	29	R	40.21	84	+	None
4	F	42	RC	70.47	120	+	None
5	F	44	RC, R, A	78.15	53	+	None
6	M	30	RC, A	81.37	75	+	None
7	M	37	R, A	72.76	53	+	None
8	M	50	RC	9.66	50	+	None
9	F	41	R	12.84	84	+	Olive species, wall pellitory species, peach, mite, dog
10	M	19	RC	0.75	22	—	Olive species, mugwort species, mite, nuts
11	F	24	RC	46.71	24	+	Olive species, weed
12	M	30	RC, R, A	29.07	156	+	Grass, wall pellitory species, cat, mite
13	M	36	U	2.95	34	+	Mite
14	M	27	RC	15.58	57	+	Grass, olive species, mite
15	M	26	R	55.43	226	+	Grass
16	M	26	RC, A	8.77	69	+	Grass, peanut, cat, dog
17	M	20	RC, A	0.72	124	—	Grass, olive species, English plantain species, cat
18	M	20	RC	16.35	102	+	Grass, olive species, English plantain species, cypress species
19	F	66	R, A	0.70	64	—	Grass, olive species, weed
20	F	14	R, A	3.56	30	+	Grass, olive species, Cypress species, dog
21	M	33	R	0.76	54	—	Grass, olive species, Cypress species, weed
22	F	23	RC	15.05	41	+	Grass, mugwort species
23	M	25	RC, A	2.74	71	+	Grass, English plantain species, olive species
24	M	30	RC	4.50	69	+	Grass, English plantain species, Cypress species
25	F	32	RC	2.98	35	—	Grass, English plantain species, Cypress species
26	M	22	RC, A	68.03	106	+	Grass, olive species, English plantain species, cypress species

SPT, Skin prick test; RC, rhinoconjunctivitis; A, asthma; R, rhinitis; U, urticaria.

RESULTS

Isolation and partial amino acid sequencing of Pla a 2

Preliminary data with immunoblotting assays showed a protein band in the *P acerifolia* extract at 43 kd, which was recognized by 83% of patients' sera.⁵ Crude pollen extract of *P acerifolia* was applied to a cation exchange column for a first enrichment in basic proteins. IgE-reactive fractions, appearing at 0.2 mol/L NaCl and containing 2 allergenic proteins of 18 and 43 kd, were pooled, concentrated, and applied to a Superdex 200 column. After chromatography, isolated protein reacted to IgE and showed, after SDS-PAGE, a single band with an

apparent molecular mass of 43 kd under reduced conditions (Fig 1, A) and 39 kd under nonreduced conditions (data not shown). The apparent molecular mass on gel filtration chromatography of purified protein was 38 ± 3 kd, suggesting a monomeric nature of the allergen. The final yield of the isolated *P acerifolia* allergen was 0.7% to 0.9% of the pollen extract. IEF of purified Pla a 2 showed a single band at an isoelectric point of greater than 9.3, which reacted to a pool of sera from patients allergic to planetree species (Fig 1, B). The purified Pla a 2 contained glycan groups because after treatment of transferred protein with metaperiodate, digoxigenin was covalently bound to the aldehyde groups and detected by using an anti-digoxigenin—alkaline phosphatase conjugate (Fig 1, C).

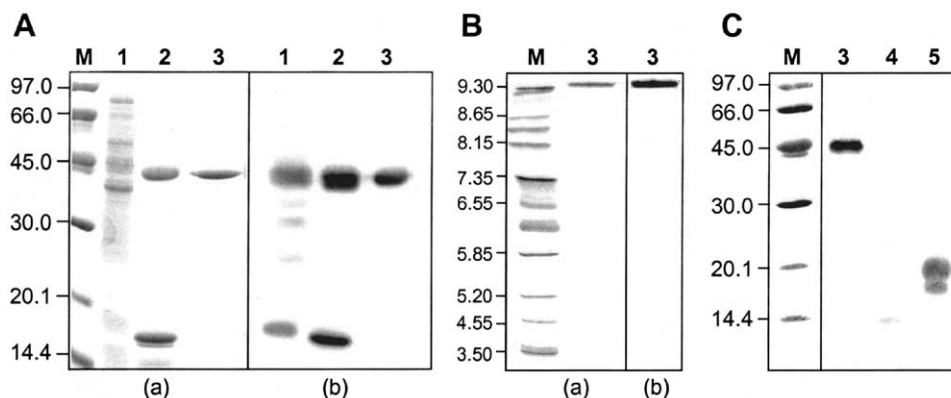


FIG 1. Electrophoretic analysis of purified Pla a 2. **A**, Coomassie-stained SDS-PAGE (a) and immunostaining with reactive sera (b) of fractions from the purification steps: raw extract (lanes 1), after the HiTrap SP column (lanes 2), and purified Pla a 2 (lanes 3). **B**, Coomassie-stained agarose IEF (a) and IgE immunoblotting (b) of purified Pla a 2. **C**, Staining of purified Pla a 2 with the Glycan Detection Kit. As controls, Ole e 2 (lane 4) and Ole e 1 (lane 5) were used.

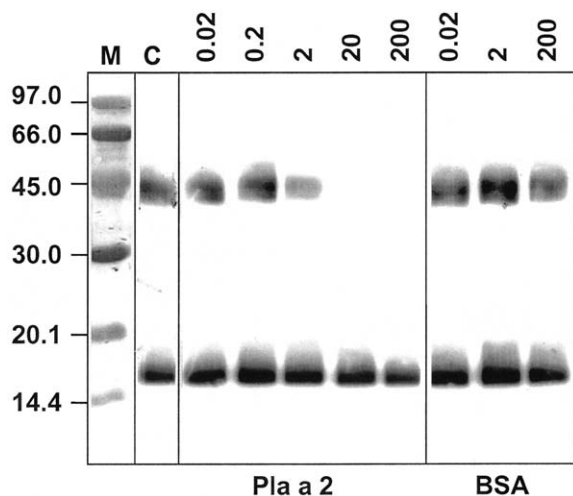


FIG 2. SDS-PAGE immunoblotting inhibition of *P acerifolia* pollen extract. Serum pool of monosensitized patients preincubated with buffer alone (C) or different concentrations (in nanograms per milliliter) of purified Pla a 2 and BSA.

The high degree of purification obtained with Pla a 2 allowed the N-terminal amino acid sequencing of its 20 first residues. Because Pla a 2 showed high resistance to trypsin digestion, chemical cleavage with CNBr was performed, and amino acid sequences of 2 internal peptides were determined by means of Edman degradation. The alignment of these sequences with those contained in the GenBank/EMBL database revealed significant homology with PG.

Enzymatic activity of Pla a 2

The potential PG activity of Pla a 2 was measured by using pectin as a substrate. Purified Pla a 2 exhibited a specific activity of 1.80 ± 0.18 (average $\pm$ SE) micromoles of glucuronic acid release per minute per milligram

of protein. The optimum pH and temperature for the activity were found to be 4.0 and 50°C, respectively.

Immunochemical characterization of Pla a 2

Immunoblot-inhibition experiments were performed by means of preincubating purified Pla a 2 with pooled sera from the 8 patients monosensitized to planetree pollen to demonstrate that purified Pla a 2 maintained immunologic properties the same as those of the pollen in the crude extract. Pla a 2 concentration of 20 ng/mL completely inhibited the IgE binding to the 43-kD protein present in the *P acerifolia* extract (Fig 2). No inhibitory effect was detected in control experiments with BSA as control inhibitor.

The capacity of isolated Pla a 2 to bind specific IgE after the purification process was evaluated by means of direct ELISA in which the purified allergen was adsorbed to the plate. Twenty-two (84.6%) of the 26 sera analyzed had specific IgE to Pla a 2. The allergenic activity of Pla a 2 was estimated by means of inhibition ELISA. Pla a 2 could strongly inhibit the binding of specific IgE to the whole extract up to 52%, as shown in Fig 3.

Cloning and characterization of primary structure of Pla a 2

On the basis of the amino acid sequences of the Pla a 2 internal peptides and a conserved sequence for PG, a nucleotide sequence of the full-length gene encoding for Pla a 2 was cloned and sequenced. The cDNA has 1395 bases, including the poly(A⁺) tail, and predicts an open reading frame of 372 amino acids in length. Because the Pla a 2 cDNA clone contains all peptide sequences obtained from chemical cleavage of Pla a 2, it certainly encodes the purified allergen (Fig 4). The deduced amino acid sequence showed 2 potential N-glycosylation sites and an expected molecular mass of 39,237 d, which agreed with that obtained after SDS-PAGE under nonreduced conditions and gel permeation. A predicted isoelectric

point value of 9.01 agreed with the basic characteristics of Pla a 2 in IEF and ionic chromatography. A predictive analysis of secondary structure of Pla a 2, performed on the basis of the amino acid sequence, showed that Pla a 2 belongs to all β -class proteins (55.1% β -sheet, 3.5% α -helix, and 41.4% loop). Accessibility prediction results showed that 54.8% of the residues presented a predicted accessibility of greater than 16% of their surface.

A protein homology search with BLAST software demonstrated that Pla a 2 shares 56%, 49%, 48%, and 43% identical residues with PGs of pollen from *Oenothera organensis* (evening primrose), *Brassica napus* (rape), *Gossypium hirsutum* (American cotton), and *Zea mays* (maize), respectively (Fig 5). Homology was also found to PGs involved in the ripening of peach, kiwi, apple, and tomato fruits (42%, 39%, 38%, and 37% identical residues, respectively). PG from Japanese cedar pollen, allergen Cry j 2, has only 34% identical amino acids with Pla a 2.

Pla a 2 and all these proteins have 6 conserved Cys residues that should be involved in the tertiary structure stability. Homology modeling of the 3-dimensional structure of Pla a 2 yielded a conformation that was highly similar to *Erwinia carotovora* PG structure.¹⁵ The crystal structure of this PG folds into a right-handed parallel β -helical structure, in which the β -helix is formed by 4 parallel β -sheets and compacted by 4 disulfide bridges (data not shown).

Expression of Pla a 2 in planetree tissues and other pollen species and plant-derived foods

The expression of Pla a 2—like proteins in other *P. acerifolia* tissues and different pollens and fruits was studied by means of immunoblotting with specific rabbit antiserum against Pla a 2. Incubation with Pla a 2 antiserum revealed specific IgG-reactive bands of 43 and 53 kD in pollen and stem extracts from *P. acerifolia* but not in leaves (data not shown). Reactive bands of 43 kD in pollen extracts from cupressaceae (*Cupressus arizonica*, *Juniperus communis*, and *Thuja plicata*) and 44 kD in tomato extracts have been found. Other reactive bands in grass pollen and fruit extracts from peach, avocado, and kiwi disappeared after treatment with periodate (data not shown).

DISCUSSION

Large quantities of airborne planetree pollen are detected in many cities of the United States and Europe,^{3,4} and high prevalence, ranging from 5% to 56%, of positive skin prick test responses to *Platanus* species has been reported in Spain.⁴ Two proteins have been described as major and specific allergens from *P. acerifolia* pollen: Pla a 1 is an 18-kD protein with a prevalence of 84.3%, representing around 60% of the specific IgE binding of monosensitized patients with *P. acerifolia* allergy and shows homology to proteinaceous invertase inhibitors but without known physiologic

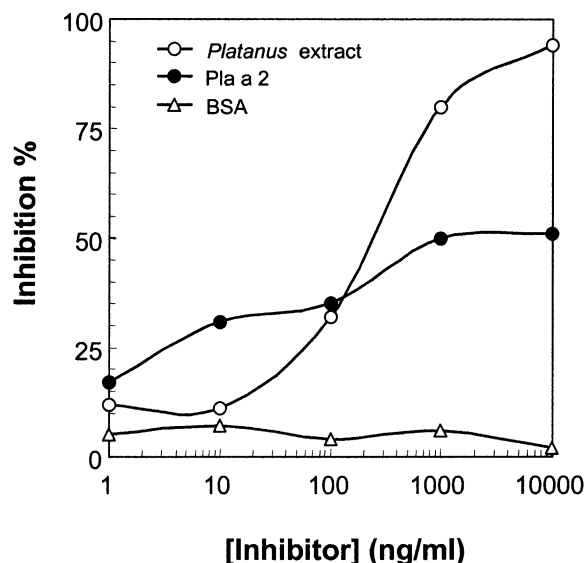


FIG 3. ELISA-inhibition curves. The binding of specific human IgE to *P. acerifolia* extract was inhibited by different concentrations of Pla a 2, *P. acerifolia* extract, or BSA as a control.

function. The other allergen is a 43-kD protein with a prevalence of 83%.^{5,7} This allergen has been now isolated and characterized as a PG.

PGs are widely distributed in plants, fungi, and bacteria, and they act as pectin-degrading enzymes. The disassembly of pectin accompanies many stages of plant development, but the majority of research has focused on PGs in ripening fruit, abscission zones, and pollen.⁸ A relatively small number of pollen allergens have been biochemically characterized as active enzymes, including 1,3- β -glucanase (Ole e 9), polymethylgalacturonase (Cry j 2), pectate lyases (Cry j 1, Cup a 1), and cyclophilin (Bet v 7).¹⁶⁻¹⁹ Alignment of Pla a 2 with PG sequences reveals high conservation of regions involved in the catalytic sites of these enzymes, and furthermore, we have also established that Pla a 2 is a functionally active PG. This is the first time PG activity of an angiosperm allergen has been demonstrated. The functional role of a PG in pollen tubes might be acting on its own wall to facilitate growth and degrading the walls of the stylar cells to allow penetration of the pollen tube.⁸

A phylogenetic tree generated from the alignment of cloned plant PG sequences groups them into 3 clades.⁸ Clades A and B are composed of genes expressed in fruit and abscission zones, whereas clade C is composed entirely of genes expressed in pollen. Pla a 2 has significant homology with members of clades A and B (42% to 37% identical residues), and as expected, it showed higher homology with members of clade C (56% to 42% identical residues). Preliminary molecular modeling yielded a Pla a 2 model with 8 right-handed, parallel β -helix domains. Pla a 2 and, in general, the parallel β -helix proteins are structures with high accessibility allowing similar antigenic properties.^{2,20} Therefore immunoblotting experiments with antiserum against Pla a 2 showed

CGCGGGTCCAGTCTAGTGGTAGTGTTCATGTAATGATTATGGTGCAAAGGGTGGTGGTATATCAGCCAGGCTGTGATGAAAGCT 90
 R G V Q S ↑ S G S V F N V N D Y G A K G A G D I S Q A V M K A 25
 TGGAAGGCTGCATGCGCATCACAGGACCAAGCACAGTCTGATTCCTAAAGGGAACATAATATGGGTGAGGTAGCTATGCAGGGACCA 180
 W K A A C A S Q G P S T V L I P K G N Y N M G E V A M Q G P 55
 TGCAAGGGTTCAAAGATTGGGTTTCAGATTGACGGGGTGGTGAAGGCTCCGGCCGATCCAAGCAAGTTCAAATCGGACGGTGGGTCTCT 270
 C K G S K I G F Q I D G V V K A P A D P S K F K S D G W V S 85
 TTCTACCGCATCGATGGCTTGACAGTCTCAGGAACAGGAACATTAGATGGTCAAGGACAAACGGCTTGGGCTAAGAACAATTGTGACAAG 360
 G T G T L D G Q G Q T A W A K N N C D K F Y R I D G L T V S 115
 AATCCAAATTGCAAGCATGAGCCATGAATTTGAGATTGAGCTTCTCTCAAACACGCCATGGTCCGGGACATAACCTCCCTGAACAGTAAA 450
 N P N C K H A A M N L R F D F L K H A M V R D I T S L N S K 145
 ATGTTTTCACATTAACTGTTGGAGGTGTGAGGATTAACCTTTCCAACACGTTACTGTACCGGCACCGGGAACAAGCATCAACACCGACGGA 540
 M F H I N V L E C E D I T F Q H V T V T A P G T S I N T D G 175
 ATCCACGTCGGAATTTCAAAGGGGGTTACTATTACTAATACCAAAATAGCCACCGGCGACGACTGCATCTCCATTGGTCTCAGGACGCCAA 630
 I H V G I S K G V T I T N T K I A T G D D C I S I G P G S Q 205
 AATGTAACCATCACCCAGGTAAACTGTGGACCGGGGCACGGCATCAGCATCGGTAGCCTTGGCAGGTATAATAATGAAAAAGAAGTACGT 720
N V T I T Q V N C G P G H G I S I G S L G R Y N N E K E V R 235
 GGGATAACAGTGAAGGGCTGCACCTTCTCCGGCACGATGAACGGGGTGAAGGTCAAGACATGGCCTAACAGTCTCTCTGGTGTGCAACC 810
 G I T V K G C T F S G T M N G V R V K T W P N S P P G A A T 265
 GATCTCACATTTCAAGATCTTACTATGAACAATGTCCAAAATCCAGTCATCTTGGATCAAGAGTATTGCCCATATGGACAGTGCACCCG 900
 D L T T F Q N D L T M N N V Q N P V I L D Q E Y C P Y C P G C S R 295
 CAGGCTCCATCGAGGATTAAGCTCAGCAACATTAACTTCAACACATTCGAGGCACCTCCACAGGCAAGGTGTCAGTCGTAATCGCGTGT 990
 Q A P S R I K L S N I N F N N I R G T S T G K V A V V I A C 325
 AGCCATGGCATGCCGTGCTCGAACAATGAAGTAGGCGAGATCAACTTGTCTGACCGTGGAGCAGGAGGGCCTGCTACCTCCACTTGTCT 1080
 S H G M P C S N M K I G E I N L S Y R G A G G P A T S T C S 355
 AACGTCAAGCCACATCTCTGGCAAACAGGTGCCAGCTATTAAATGTGCCCTAATTGCCATTAGGGTTGTATAAATTTCTAGTATTTCT 1170
 N V K P T F S G K Q V P A I K C A * 372
 GTGATAATAAGGTATAGGGTTATCTTACATTTTGTAAAGGAGAGAGATTGTTCCACAACGTAGACCCCTCCGGCGAGAAGCCAACCAT 1260
 GAGGGTCATCAACTAGCTCAGGTTGTTGTTTCGATGTCATAAGTCCCTAAAGGACGATTGCTGTAAGGGCCAGGCACCCCTTTTCTCTT 1350
 AAGTTTTGATGAAACTTCAATTAGCAATGAAAAAATAAAAAA 1395

FIG 4. Sequence analysis of cDNA encoding Pla a 2: nucleotide and deduced amino acid sequences. Sequenced internal peptides are underlined, and predicted N-glycosylation sites are double underlined. The arrow indicates the experimental cleavage site for signal peptide. The nucleotide sequence for Pla a 2 has been deposited in the GenBank database under accession no. AJ586898.

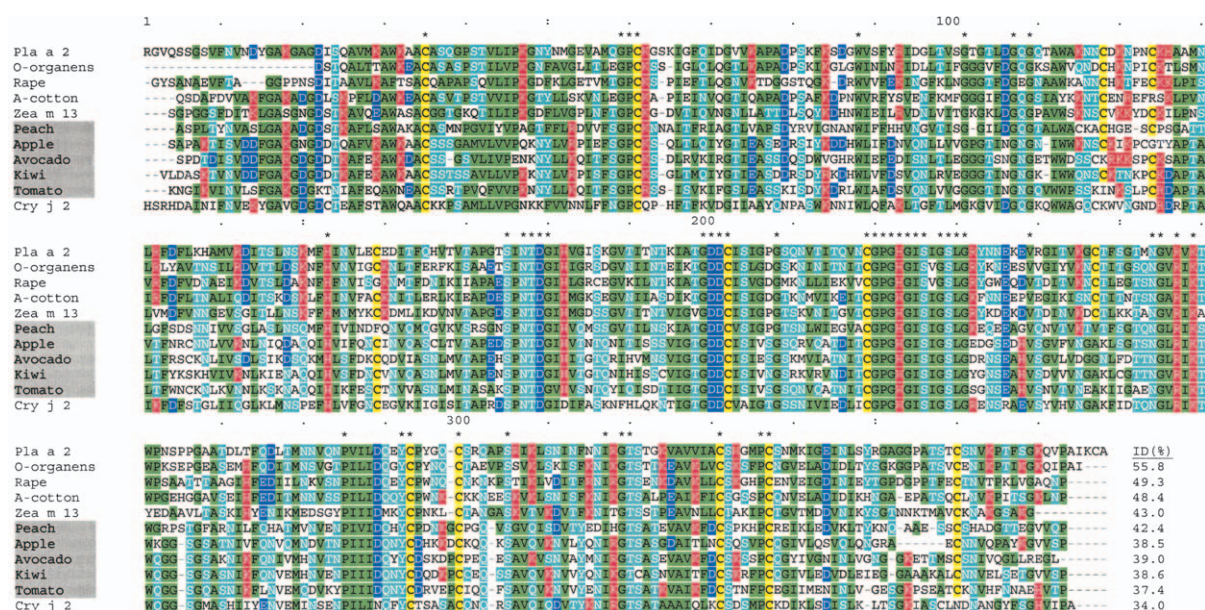


FIG 5. Pollen and fruit PG amino acid sequence alignment (accession nos. AJ586898, P24548, P35337, Q39786, P35339, P48979, P48978, Q02096, P35336, P05117, and P43212, respectively). Asterisks represent conserved residues. Amino acids were colored to illustrate conservation of physicochemical properties: yellow, conserved; light green, hydrophobic; light blue, hydrophilic; dark blue, acidic; red, basic.

cross-reactivity with PGs from tomato or pectate lyase (cypress group 1), both of which have been described as important allergens.^{18,21}

Regarding the importance of the new allergen described here, ELISA with purified Pla a 2 indicated that it is recognized by more than 84% of patients allergic to *P acerifolia* pollen. Furthermore, Pla a 2 represents about 52% of the total IgE-binding capacity of London planetree extract. Pla a 2 has high homology with PGs from pollen and plant foods, including known pollen allergens, such as group 13 from grass,²² the 43-kd allergen from oilseed rape,²³ Cry j 2 from Japanese cedar,²⁴ and the 46-kd allergen from tomato.²¹ Studies on T-cell and B-cell epitopes have been performed in the Japanese cedar pollen allergen Cry j 2.^{25,26} Five T-cell and one dominant B-cell epitopes have been described for this allergen, but none of them have homologous sequences in Pla a 2. The broad distribution of these carbohydrate-degrading enzymes in pollen and fruits suggests that similar to panallergen profilin, IgE binding to higher-molecular-weight components might be explained, at least in part, by the existence of PGs. The existence of a high degree of conservation in the primary structure among PGs agrees with the cross-reactivity observed between *P acerifolia* and plant-derived foods.²⁷ Nevertheless, the exact meaning of these results should be further investigated by using sera from patients allergic to these pollens and plant-derived foods. It has been recently described that oral allergy syndrome in some patients might have been caused by primary respiratory sensitization to *P acerifolia*,²⁷ and food allergens involved in the oral allergy syndrome of these patients were nuts, peach, kiwi, and maize, which could contain Pla a 2—like proteins. Therefore the involvement of Pla a 2 in this primary sensitization can not be ruled out and must be studied in detail.

In conclusion, the study identifies and characterizes Pla a 2 as a relevant allergen in *P acerifolia* pollen with functional PG enzyme activity. The contribution of this allergen and Pla a 1⁷ to the specific IgE binding of mono-sensitized patients with *P acerifolia* allergy suggests the existence of a 2-allergen hypersensitivity model, which could indicate *P acerifolia* as an ideal example for diagnosis and immunotreatment with recombinant allergens.

We thank the Departamento de Química de Proteínas (Centro de Biología Molecular, Madrid) and the Servicio de Secuenciación (Centro de Investigaciones Biológicas, Madrid) for amino acid and DNA sequencing facilities.

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