

Identification of a novel hydroxyproline-rich glycoprotein as the major allergen in *Parthenium* pollen

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Background: The airborne pollen of the Compositae weed, *Parthenium hysterophorus*, is a major cause of allergic rhinitis in the Indian subcontinent and in certain parts of the southern United States and western Australia. Earlier studies have identified a 31 kd protein as the major allergen in *Parthenium* pollen.

Objective: This study was undertaken to carry out the purification, immunochemical characterization, sequencing, and epitope analysis of this major allergen, designated as Par h 1.

Methods: The IgE-binding activity of the allergen was evaluated by immunoblot and inhibition ELISAs. Pronase digestion, periodate oxidation, and chemical deglycosylation were performed to determine the role of peptide and carbohydrate components of the allergen in IgE binding.

Results: The data provide evidence for the involvement of carbohydrate moieties on Par h 1 in its IgE-binding ability. The N-terminal 91 amino acid sequence of Par h 1 shows 81% identity with a protein from sunflower anther, and the hydroxyproline-rich region of Par h 1 is 30% to 40% identical to similar stretches in extensins, a class of hydroxyproline-rich cell wall glycoproteins from different plant species. IgE antibodies in the sera of individuals allergic to *Parthenium* cross-reacted with a 50 kd hydroxyproline-arabinose-rich extensin precursor from potato tuber, and this binding was periodate-sensitive.

Conclusions: It appears that a group of soluble plant glycoproteins, which are related to the ubiquitous extensins, have certain carbohydrate-containing IgE-binding epitopes that may contribute to allergenic cross-reactivity among specific pollens and foods. (*J Allergy Clin Immunol* 1996;98:903-12.)

Key words: *Parthenium*, glycoprotein, allergen, hydroxyproline

Inhalant allergens including pollens, dust mites, animal dander, and fungal spores contribute to the genesis of immediate hypersensitivity reactions of the airways. Pollen from the Compositae weed, *Parthenium hysterophorus*, has been identified as an important aeroallergen and a major cause of aller-

Abbreviations used

CBB:	Coomassie Brilliant Blue
HPLC:	High-performance liquid chromatography
PAS:	Periodic acid-Schiff
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
TFMS:	Trifluoromethanesulfonic acid

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gic diseases, including allergic rhinitis, in different parts of India¹⁻³ and the southwestern United States.⁴ *Parthenium* pollen allergens also cross-react with those from ragweed, another member of the Compositae family.⁵ The rapid onset of symptoms of allergic rhinitis on exposure to *Parthenium* pollen, observed in *Parthenium*-sensitive individuals,³ correlates with the rapid release of allergens from *Parthenium* pollen in vitro.⁶

Immunoblot analysis of crude extracts of *Parthe-*

nium pollen has identified a number of allergens in the molecular weight range of 14 to 70 kd.² Periodate-Schiff staining and lectin overlay experiments have revealed that the allergens are glycoproteins. *Parthenium* pollen extract has also been reported to lose its ability to bind IgE antibodies after mild periodate oxidation, whereas extensive proteolysis had no appreciable effect.⁶ Immunoscreening of a crude extract of *Parthenium* pollen with sera from *Parthenium*-sensitive patients revealed that the most significant IgE-binding activity, in more than 90% of sera tested, was associated with a 31 kd glycoprotein.^{3,6} In this study we report the purification and immunochemical characterization of this 31 kd allergen, designated as Par h 1. We also examine the chemical nature of its allergenic epitopes by primary structure analysis and cross-reactivity experiments.

METHODS

Sera

Serum samples were obtained after informed consent was obtained from patients with allergic rhinitis, a positive skin test response to *Parthenium* allergen extract, and elevated but comparable levels of *Parthenium*-specific serum IgE. Sera were stored as 1 ml aliquots at -90°C . A serum pool was generated by mixing equal volumes of sera from six patients who had *Parthenium*-specific IgE antibodies.

Purification of the 31 kd allergen from *Parthenium* pollen

One gram of dry pollen, collected and processed as previously described,³ was extracted with 10 ml of 10 mmol/L sodium acetate buffer, pH 5.2 (extraction buffer), for 16 hours at 4°C with end-to-end mixing. The extract was clarified by centrifugation at 20,000 g for 15 minutes at 4°C , and the supernatant was loaded onto a DEAE-Sepharose CL-6B anion exchange column (15 ml bed volume; Pharmacia Fine Chemicals, Uppsala, Sweden). After washing with extraction buffer, the bound allergen was eluted by applying a linear gradient of 0 to 0.2 mol/L NaCl in extraction buffer. The elution peaks were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for the presence of the 31 kd IgE-binding protein; and fractions showing IgE-binding activity were pooled, dialyzed against distilled water, concentrated, and subjected to high-performance liquid chromatography (HPLC) with a Shimpack Diol-150 column and a Shimadzu LC-6A HPLC system (Shimadzu Corp., Kyoto, Japan). Distilled water was used as the mobile phase at a flow rate of 1 ml/min, and the elution profile was monitored at 280 nm. The major allergen eluted as a single peak with a retention time of 7.2 minutes. The purified allergen (500 μg) obtained was designated as Par h 1.

Evaluation of IgE binding activity in vitro

The IgE-binding activity of each allergen preparation was assessed by an avidin-biotin microtiter ELISA as previously described,^{3,7} except for the use of a 1:500 dilution of anti-human IgE-biotin conjugate.

Analytic methods

The protein and carbohydrate measurements were performed by using Bradford's dye binding assay⁸ and a phenol sulfuric acid method,⁹ respectively. Electrophoresis was performed on SDS-containing 15% polyacrylamide gels with Laemmli's discontinuous buffer system.¹⁰ The separated proteins were visualized by staining with silver or Coomassie Brilliant Blue R-250 (CBB) (Bio-Rad Laboratories, Hercules, Calif). Chemical detection of carbohydrate after SDS-PAGE was accomplished by periodic acid oxidation, followed by reaction with Schiff reagent.¹¹ Isoelectric focusing was performed on a 5% polyacrylamide gel between pH 3.5 and 10, at 2000 V and 50 mA, according to the manufacturer's instructions (Pharmacia Fine Chemicals). The gel was calibrated with standard marker proteins (isoelectric point range, 4.5 to 9.6).

Immunoblotting of allergens

Proteins separated by SDS-PAGE were transferred onto nitrocellulose.¹² Blots were then blocked with phosphate-buffered saline, pH 7.4, containing 0.5% Tween-20 and 2% goat serum (blocking buffer) for 1 hour at 37°C and incubated overnight at 4°C with the sera of patients with *Parthenium*-induced rhinitis, diluted appropriately in blocking buffer. The strips were washed with 0.05% Tween-20 in phosphate-buffered saline, pH 7.4 (rinse buffer), and incubated with anti-human IgE-biotin conjugate (diluted 1:500) for 2 hours at 37°C . After washing with rinse buffer, the nitrocellulose strips were incubated with avidin-horseradish peroxidase (diluted 1:2000) for 1 hour at 37°C , washed again with rinse buffer, and placed in peroxidase chromogenic substrate solution (1 mg/ml 3,3'-diaminobenzidine and 0.03% H_2O_2 in 50 mmol/L citrate-phosphate buffer, pH 5.5). The individual IgE-binding components were identified by the activity of the bound enzyme. In immunoblot inhibition experiments, the serum was exposed to inhibitor at 37°C for 1 hour and then incubated with the blots.

Pronase digestion

The purified allergen was dialyzed at 4°C against 100 mmol/L Tris-HCl buffer, pH 8.0, containing 10 mmol/L CaCl_2 . Pronase E (Sigma Chemical Co., St. Louis, Mo.), predigested for 2 hours at 37°C to destroy any contaminating glycosidases or other interfering enzyme activities, was added to the allergen (enzyme to protein ratio of 1:10) and incubated at 45°C under toluene.¹³ A fresh aliquot of pronase was added after every 24 hours, and proteolysis was terminated at 96 hours by immersing the digestion mixture in a boiling water bath for 5 minutes. The proteolytic digest was centrifuged, and the residual

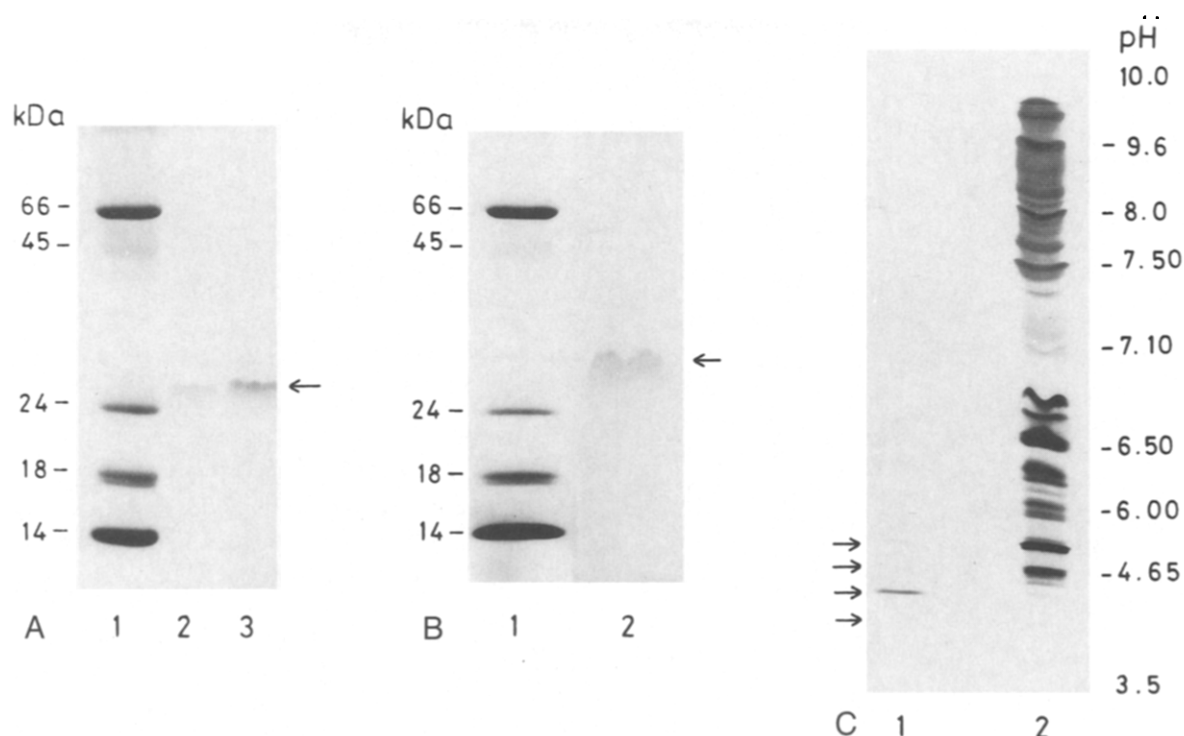


FIG. 1. Biochemical characteristics of Par h 1. Purified Par h 1 was run on an SDS-containing 15% polyacrylamide gel, and resolved (glyco)proteins were visualized by staining with **(A)** CBB (lane 1, molecular weight markers; lanes 2 and 3, 250 ng and 500 ng Par h 1) and **(B)** PAS reagent (lane 1, molecular weight markers; lane 2, 5 µg Par h 1. Par h 1 (500 ng) was subjected to isoelectric focusing on a 5% acrylamide gel in the pH range of 3.5 to 10, and the proteins were detected by staining with **(C)** CBB (lane 1, Par h 1; lane 2, standard isoelectric point markers).

IgE-binding activity of the supernatant was evaluated by SDS-PAGE, immunoblot, and inhibition ELISA.

In situ periodate oxidation

Par h 1 (5 µg/ml) was coated onto polystyrene microtiter wells (100 µl/well) for 1 hour at 37° C. After washing twice with 0.1 mol/L sodium acetate buffer, pH 6.0, the allergen-coated wells were incubated with 20 mmol/L sodium *meta*-periodate in the same buffer for 4 hours in the dark at room temperature. Wells incubated with buffer alone served as controls. The wells were washed five times with rinse buffer and blocked with blocking buffer for 1 hour at 37° C. The remaining steps of ELISA were carried out as described previously.

Chemical deglycosylation in situ

Par h 1 was subjected to SDS-PAGE, the gel was stained with CBB for 30 minutes, and the band corresponding to Par h 1 was excised and de-stained. One gel slice was immersed in 500 µl of trifluoromethanesulfonic acid (TFMS) (Sigma Chemical Co.), and another was immersed in 500 µl of distilled water and incubated on ice for 12 hours. The gel slices were then washed extensively with distilled water, equilibrated with SDS-PAGE tank buffer (0.025 mol/L Tris, 0.192 mol/L glycine, 0.1% SDS, pH 8.3),¹⁰ and loaded into adjacent

wells of an SDS-containing 15% polyacrylamide gel. The protein and glycoprotein bands were visualized by silver or periodic acid-Schiff (PAS) staining, respectively. To assess the IgE-binding ability of the TFMS-treated allergen, the separated protein was transferred onto nitrocellulose membranes, and immunodetection was carried out as described previously.

Amino acid analysis

Par h 1 was subjected to hydrolysis in vapor phase with constant boiling HCl for 1 hour at 160° C by using a Pico-Tag work station (Waters Associates, Boston, Mass.). The analysis was performed on a Beckman amino acid analyzer model 6300 and quantitation with System Gold Software (Beckman Instruments, Inc., Fullerton, Calif.). The amount of hydroxyproline was estimated from absorbance at 440 nm for Asp, because both hydroxyproline and aspartate co-elute at the same place. From one run done at another site, an estimate of hydroxyproline to Asp was 8.5:1, as determined by physiologic analysis.

Amino acid sequencing

The N-terminal analysis of Par h 1 was performed by Edman degradation. Par h 1 was subjected to digestion with endoproteinase Lys C (Boehringer Mannheim GmbH, Mannheim, Germany) with or without reduc-

TABLE I. Amino acid composition of Par h 1

Amino acid	No. of residues (Integral values)
Aspartic acid	29.0*
Threonine	10.0
Serine	10.1
Glutamic acid	32.3
Proline	9.2†
Glycine	123.9
Alanine	32.6
Valine	4.1
Methionine	0.1
Isoleucine	2.0
Leucine	4.5
Phenylalanine	7.7
Tyrosine	2.8
Histidine	3.3
Lysine	28.0
Arginine	5.6
Tryptophan‡	ND
Cysteine	3.7

The integral values were calculated assuming the molecular weight of Par h 1 to be 31 kd by SDS-PAGE.

ND, Not determined.

*Because Asp and OH-Pro elute at the same retention time, neither amino acid can be quantitated individually. By physiologic analysis of one of the runs, and estimate of 8.5:1 of OH-Pro:Asp was achieved. Therefore the integral value for Asp is approximately 3-4, with 25-30 OH-Pro residues.

†The concentration of proline was determined by analysis with a 440 nm channel.

‡The number given is for half-cysteine.

tion/alkylation of the disulfides in 100 mmol/L Tris-HCl buffer, pH 8.0, at 37° C for 1 hour (enzyme to protein ratio of 1:50). The digestion products were separated on a C4 Vydac reverse-phase HPLC column (Vydac, The Separations Group, Hesperig, Calif.) with a gradient of 0% to 100% solvent B (solvent A: 0.12% trifluoroacetic acid in distilled water; solvent B: 0.1% trifluoroacetic acid in 70% acetonitrile). Individual peaks were purified and sequenced by using an Applied Biosystems Model 470A gas phase sequenator with an on-line PTH analyzer (Applied Biosystems, Division of Perkin-Elmer Corp., Foster City, Calif.).

Computer predictions

The partial amino acid sequence of Par h 1 was compared for homology with known sequences in the SWISS-PROT protein sequence database (release 19).

Enrichment of 50 kd lectin from potato tuber

The 50 kd hydroxyproline-rich lectin was enriched from potato tubers according to the procedure of Allen and Neuberger.¹⁴ Briefly, potatoes were peeled, washed, cut into small pieces, and homogenized in 2 volumes of

0.1 mol/L sodium acetate buffer, pH 3.8, containing 2 mmol/L sodium metabisulfite as antioxidant. The homogenate was centrifuged at 600 g for 15 minutes, and the supernatant was filtered through cheesecloth. The residue was resuspended in the same buffer, and the procedure was repeated twice. Ammonium sulfate was then added to the combined supernatants to achieve 50% saturation, and the mixture was allowed to stand overnight at 4° C. The precipitate was then collected by centrifugation at 9000 g for 20 minutes, resuspended in distilled water, and dialyzed extensively against the same. This enriched lectin preparation was used in immunologic cross-reactivity experiments.

RESULTS

Immunochemical properties of Par h 1

The major allergen (designated as Par h 1) was purified from the pollen of *Parthenium hysterophorus* by using a combination of DEAE-Sepharose anion-exchange chromatography and HPLC on a Shimpack Diol-150 gel filtration column. The purified protein was found to be homogenous on SDS-PAGE (Fig. 1, A) and revealed a single N-terminal amino acid (lysine) on Edman degradation. Its molecular weight was estimated to be 31 kd, both by SDS-PAGE and gel filtration (data not shown). The allergen was a glycoprotein as assessed by PAS staining (Fig. 1, B). Quantitation by the phenol sulfuric acid method revealed that Par h 1 contains approximately 40% carbohydrate (wt/wt) in the form of neutral sugars (data not shown). Apparent isoallergens were observed on isoelectric focusing with isoelectric point values ranging from 4.3 to 5.2 (Fig. 1, C). Amino acid analysis revealed that Par h 1 is rich in glycine and proline, with moderate amounts of alanine and lysine along with other amino acids (Table I). The amino sugars, N-acetylgalactosamine and N-acetylglucosamine, were present in a ratio of 2:1. Par h 1 bound to IgE antibodies in the sera of *Parthenium*-sensitive individuals but not in those of normal subjects (Fig. 2, A). The specificity of binding was established by inhibition ELISA in which 0.5 µg/ml Par h 1 could inhibit the binding of *Parthenium*-specific IgE antibodies to the crude pollen extract immobilized in microtiter wells by 50% (Fig. 2, B).

Evidence for the IgE-binding capacity of protein-linked carbohydrate on Par h 1

The chemical nature of allergenic epitopes on Par h 1 was elucidated by selectively destroying either the polypeptide backbone or the covalently linked carbohydrate. For the former, Par h 1 was subjected to extensive proteolytic digestion with pronase E; and the resulting digest was analyzed by

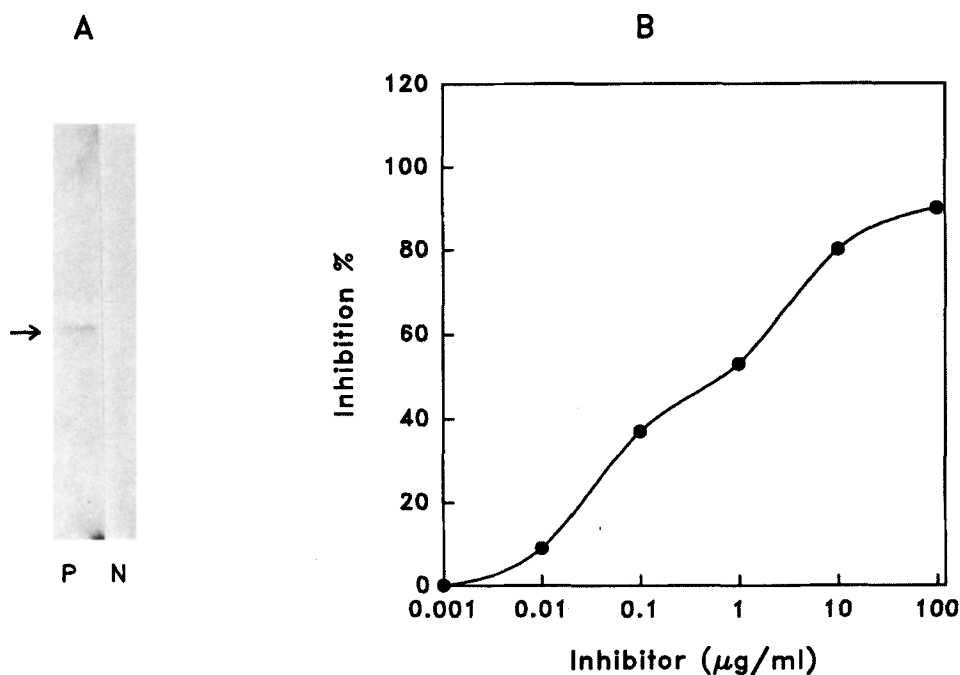


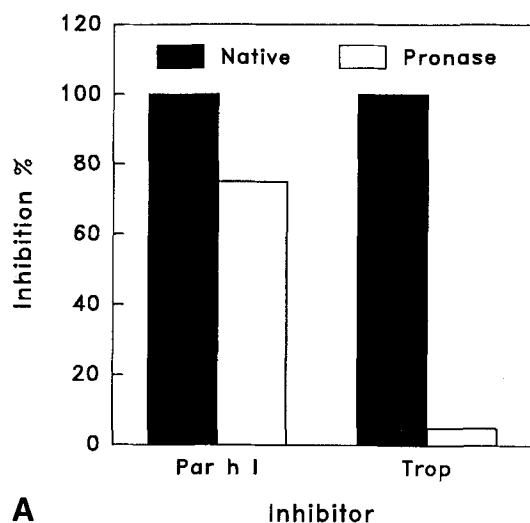
FIG. 2. Allergenicity of Par h 1. **A**, Purified Par h 1 was subjected to SDS-PAGE, and separated proteins were electrophoretically transferred onto nitrocellulose membranes. Blots were probed with pooled diluted serum (1:10) from patients allergic to *Parthenium* pollen (*P*) and normal subjects (*N*). **B**, Crude pollen extract was coated onto polystyrene microtiter plates (5 μg/ml) and probed with *Parthenium*-specific serum, which was preincubated with increasing concentrations of Par h 1 (inhibitor, 0.001 to 100 μg/ml).

SDS-PAGE, immunoblot, and inhibition ELISA experiments. No CBB staining or IgE-binding bands were observed on SDS-PAGE and immunoblotting of the pronase digest with pooled serum from *Parthenium*-sensitive patients (data not shown). Interestingly, an inhibition ELISA experiment revealed that even after 96 hours of exhaustive digestion at 45° C, the digest retained 75% of its IgE-binding activity (Fig. 3, *A*). Shrimp tropomyosin, which has been identified as the major cross-reacting crustacean allergen and which has IgE-binding epitopes that are known to be proteinaceous in nature,¹⁵ was used as a control. When tropomyosin was treated similarly with pronase E, its ability to bind to IgE antibodies in the sera of shrimp-sensitive patients was lost. In situ oxidation of the carbohydrate on Par h 1 with 20 mmol/L sodium *meta*-periodate led to a drastic reduction in its allergenicity (Fig. 3, *B*). To obtain further proof for the involvement of glycosyl moieties in allergenicity, Par h 1 was subjected to in situ chemical deglycosylation with TFMS. SDS-PAGE analysis of the native and TFMS-treated allergen did not show any difference in their protein profiles. However, the carbohydrate staining of the TFMS-

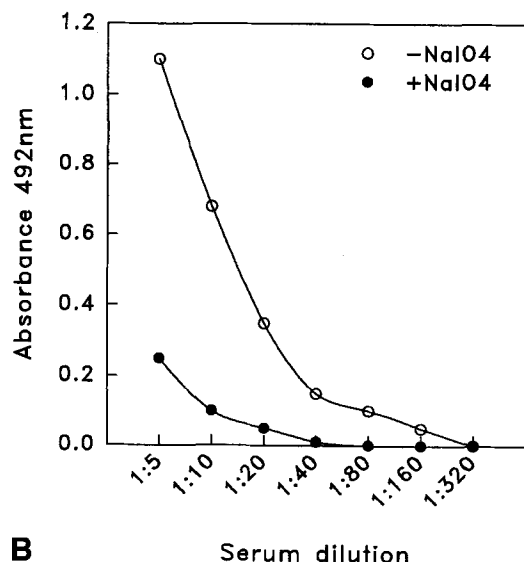
treated allergen with PAS reagent was found to be diminished with a concomitant loss in its IgE-binding capacity, as evaluated by immunoblotting (data not shown). Par h 1 was resistant to enzymatic deglycosylation, and its IgE-binding activity was not affected by treatment with N-glycanase, O-glycanase, or endoglycosidase F under appropriate conditions.

Primary structure analysis of Par h 1

To determine putative N-linked glycosylation sites, reduced/alkylated Par h 1 was digested with Lys C, and individual peptides were purified and sequenced. The resulting peptide sequences from both the native and reduced/alkylated Par h 1 allowed alignment of the sequence to yield the N-terminal 91 amino acid residues of Par h 1 (Table II). The sequence was found to possess a cysteine-rich region and a proline-rich region where most of the prolines were hydroxylated. No N-glycosylation site was predicted for the indicated stretch of the sequence. Comparison of the partial sequence of Par h 1 with known sequences in the SWISS-PROT protein sequence database revealed an 81% identity and more than 95% homology



A



B

FIG. 3. Chemical nature of allergenic epitopes on Par h 1. **A**, Polystyrene microtiter wells were coated with 5 μ g/ml Par h 1 or shrimp tropomyosin (*Trop*) and incubated with serum from a *Parthenium*- or shrimp-sensitive individual (1:10 dilution) respectively, which was preincubated with 10 μ g/ml native or pronase-digested Par h 1 or tropomyosin, respectively. **B**, Par h 1-coated microtiter wells were oxidized in situ with 20 mmol/L sodium *meta*-periodate in sodium acetate buffer, pH 6, and probed with serial dilutions of sera from a patient with *Parthenium*-induced rhinitis; wells incubated with buffer alone served as controls.

with an anther-specific cell wall protein from sunflower (*Helianthus annuus*). The cysteine- and proline-rich domains were found to be conserved between the two proteins (Table III). A 30% to 40% identity was also observed in a 30 to 40 amino acid overlap between the hydroxyproline-rich region of Par h 1 and similar stretches in a class of cell wall proteins called extensins from tobacco,

maize, sorghum, and carrot. Certain proline residues (shown in boxes) were found to be conserved between Par h 1 and the four extensins (Table IV).

Cross-reactivity of Par h 1 with a 50 kd lectin from potato

To gain further insight into the constitution of IgE-binding epitopes on Par h 1, its cross-reactivity with a 50 kd hydroxyproline-rich lectin from potato tuber¹⁴ was tested. A crude phosphate-buffered saline extract of potato tuber contained a number of polypeptides of which the 12 to 14 kd, 18 to 20 kd, and 45 to 50 kd proteins bound strongly to IgE antibodies in the sera of *Parthenium*-sensitive patients (data not shown). The 50 kd lectin was enriched from potato tubers according to a published protocol.¹⁴ The glycoprotein nature of the 50 kd lectin was confirmed by PAS staining after SDS-PAGE (data not shown). An inhibition ELISA was performed to determine the specificity of binding of *Parthenium*-specific IgE antibodies to the 50 kd lectin. Both Par h 1 and the enriched lectin inhibited the binding of IgE antibodies in the sera of patients with *Parthenium*-induced rhinitis to the enriched lectin in a dose-dependent manner (Fig. 4). Like Par h 1, in situ periodate oxidation of the enriched lectin resulted in a remarkable decrease in its ability to bind IgE antibodies (Fig. 5).

DISCUSSION

The major allergen, Par h 1, from the pollen of *Parthenium hysterophorus* has been purified and shown to be a 31 kd acidic glycoprotein, which, in spite of possessing a single N-terminal amino acid, exists as isoforms on isoelectric focusing (Fig. 1). Pronase digestion, periodate oxidation, and chemical deglycosylation data strongly suggest that the oligosaccharide sequences covalently linked to the polypeptide backbone of Par h 1 are important in binding of this allergen to IgE antibodies in the sera of *Parthenium*-sensitive subjects.

Several reports in the literature address the direct role of protein-linked carbohydrates in allergenicity. The H-antigen from the body fluid of sea squirt,¹⁶ phospholipase A₂, the major allergen of honeybee venom,¹⁷ glycoprotein 2 from ryegrass,¹⁸ the high-molecular-weight glycoprotein/polysaccharide allergens from *Pityrosporum ovale* and *Candida albicans*,¹⁹ and the differentially glycosylated isoforms of the major allergen of olive pollen²⁰ are a few examples. In this study direct evidence for the binding of sugars on Par h 1 to IgE antibodies could not be provided because attempts to deglycosylate the protein enzymatically were unsuccessful. It appears that the linkage of carbo-

TABLE II. The N-terminal 91 amino acid sequence of Par h 1 showing conserved cysteine residues and a proline-rich domain

1	K	V	<u>C</u>	E	K	P	S	K	TW	F	G	N	<u>C</u>	15
														K
16	D	T	E	K	<u>C</u>	D	K	R	<u>CM</u>	E	W	E	G	30
														A
31	K	H	G	A	<u>C</u>	H	Q	R	ES	K	Y	M	<u>C</u>	45
														F
46	<u>C</u>	Y	F	D	<u>C</u>	D	P	K	↓ KN	P	G	OH P	OH P	60
														P
61	G	A	OH P	G	T	OH P	G	T	OH PP	A	OH P	P	G	75
									OH			OH		E
76	G	E	G	C	A	OH P	P	G	XX	A	E	G	P	90
							OH						OH	A
91	G													

C, Conserved cysteine residue.

The arrow indicates the demarcation between the cysteine-rich and proline-rich domains.

hydrate to protein is extremely stable and refractory to glycosidase action. Even TFMS treatment did not result in complete deglycosylation because the carbohydrate staining for the TFMS-treated glycoprotein was not completely diminished. The IgE-binding ability of the TFMS-treated allergen, however, was lost completely, implying that only terminal sugars that could be removed on chemical deglycosylation might be involved in IgE binding (data not shown).

The N-terminal amino acid sequence of Par h 1 is rich in cysteine and proline residues, in which 12 of 15 prolines were hydroxylated. The presence of hydroxyproline is a characteristic feature of some primary plant cell wall proteins. A 25 kd protein associated with maize pollen exines has been isolated²¹ and shown to contain substantial amounts of hydroxyproline. The amino acid composition of this protein strongly implied that it was an arabinogalactan. Members of another class of hydroxyproline-rich proteins, known as extensins, are a major component of primary cell walls and are known to play a role in maintaining cell wall integrity.²² Apart from extensins, other structural cell wall proteins, such as the glycine-rich protein in *Petunia*²³ and the proline-rich proteins in carrot²⁴ and soybean,²⁵ have been reported. The vacuolar class I chitinases A and B in tobacco have recently been shown to comprise a new class of hydroxyproline-containing proteins.²⁶ In this study

the hydroxyproline-rich region of Par h 1 had a 30% to 40% identity with extensins from tobacco, maize, sorghum, and carrot in a 30 to 40 amino acid overlap (Table IV). Among allergens, the cross-reacting Group V allergens in pollens from related grass species such as ryegrass, Kentucky bluegrass, and orchard grass have been reported to contain high levels of alanine (25% to 32%) in addition to hydroxyproline.²⁷ On the basis of experimental data, it has been suggested that alkali-stable hydroxyproline-arabinoside linkages are present in the basic glycoprotein allergen from the pollen of timothy grass.²⁸ Indeed, hydroxyproline-rich glycoproteins are extensively modified by hydroxylation of proline, O-glycosylation of serine and hydroxyproline residues, and both intra- and intermolecular cross-linking. The most common substituents are short arabinoside chains and occur in every type of hydroxyproline-rich glycoprotein examined thus far including extensins, arabinogalactan proteins, gum arabic glycoproteins, repetitive proline-rich proteins, and the solanaceous lectins. It has been recently discovered that hydroxyproline arabinosylation is not random but correlated with the contiguity of the hydroxyproline residues.²⁹ The amino acid composition of Par h 1, the presence of dipeptidyl hydroxyproline residues in its partial sequence, and its ability to resist enzymatic deglycosylation with N-glycanase, O-glycanase, and endoglycosidase F strongly sug-

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1
LVFVVAISDIATVNG
1
16
21
DKRCMEWEGAKHGACHQRES
-----I D -----
36
41
KYMCF CYFD CDPKKNPG P P P
-H ----- Q -----
56
61
GA P GT P GT P P A P P G E G E G C A
----- K ----- D -
76
81
P P G X X A E G P A G
-H P P P T P S - P - GDGGSGPAPPAGGGSPP
96 106 123
PAGGDGGGGAPPPAGGDGGGGAPPPAGGDGGGAPPPGA
124 161

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*The 1-91 N-terminal amino acid sequence of Par h 1 has been aligned with residues 16 to 106 of the deduced amino acid sequence of the SF18 clone³⁰ for an anther-specific cell wall protein of sunflower (*Helianthus annuus*).

<i>Parthenium</i> *	Y	F	D	C	D	P	K	K	N	P	G	P	P	G	A	P	G	T	P	G	T	P	A	P	P	G	E	G	E	G	C	A	P	P	
Tobacco†	-	S	P	P	P	-	T	Y	L	-	P	-	-	-	S	S	-	P	P	-	S	F	S	-	P	-	-	T	Y	E	Q	S	P	P	
Maize‡							-	-	P	T	-	P	T	Y	T	P	S	-	K	P	-	T	P	K	-	T	-	-	T	Y	T	P	S	P	K
Sorghum§							-	P	A	-	K	-	S	-	P	-	-	T	P	-	T	Y	-	-	-	V	S	H	T	P	S	S	P	P	P
Carrot										-	P	-	-	E	H	S	-	P	P	-	E	H	S	-	P	-	-	Y	H	Y	-	S	P	P	

||Residues 39-65 of amino acid sequence of carrot.

sunflower protein (amino acid sequence derived from the complementary DNA sequence of the SF18 clone) is known to be expressed in the late developmental stages of epidermal anther cells and apparently contributes to cell wall architecture through intermolecular disulfide cross-linking.³⁰ This homology would be consistent with the taxonomic proximity of *Parthenium* and sunflower

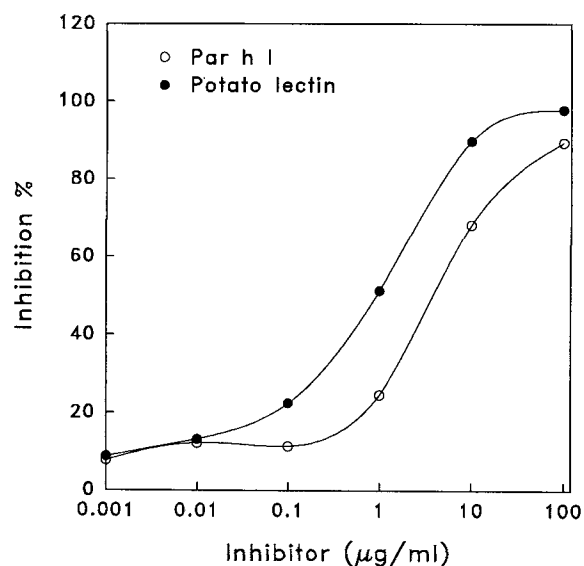


FIG. 4. Evaluation of allergenic cross-reactivity between Par h 1 and potato lectin by inhibition ELISA. A *Parthenium*-sensitive patient's serum (1:10 dilution) was preincubated with increasing concentrations of Par h 1 or potato lectin (0.001 to 100 µg/ml) before addition to microtiter wells coated with the enriched potato lectin.

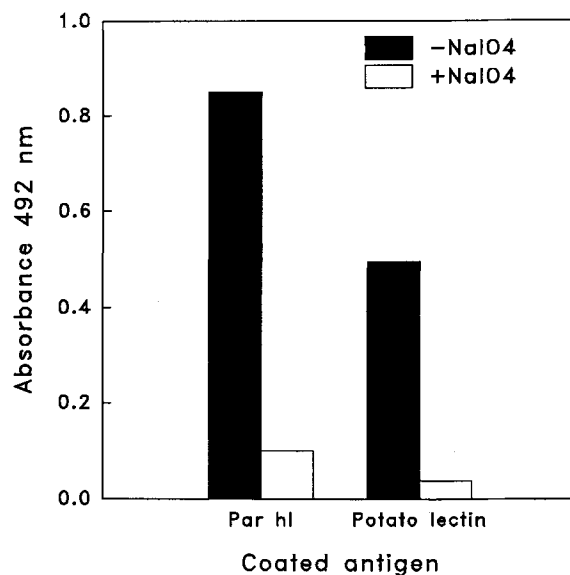


FIG. 5. Periodate-sensitive IgE-binding epitopes on potato lectin. Microtiter wells coated with Par h 1 or potato lectin (5 µg/ml) were subjected to periodate oxidation in situ, as described in Methods, and probed with serum from a *Parthenium*-sensitive individual (1:10 dilution). Wells incubated with buffer alone served as controls.

(family, Compositae; tribe, Helianthae). The calculated molecular weight of the sunflower protein is approximately 16 kd, which is equivalent to that of the protein portion of Par h 1 (18 kd), considering that 40% of the total weight (31 kd) is contributed by carbohydrates. Interestingly, clinical studies from our laboratory have revealed a high degree of allergenic cross-reactivity between *Parthenium* and sunflower pollen.³¹ Thus by virtue of the observed homology between Par h 1 and the sunflower protein and also between Par h 1 and the above-mentioned extensins, we assume that Par h 1 might also be a cell wall protein and could be playing a similar role in *Parthenium* pollen. Besides having a structural role, hydroxyproline residues in pollen have also been implicated in the stimulation of pollen germination and pollen tube elongation.³²

Further information regarding the constitution of allergenic determinants on Par h 1 can be inferred from cross-reactivity studies with a 50 kd potato lectin. The latter has been reported to contain about 50% carbohydrate, of which 92% sugar residues are arabinose and the most abundant amino acid is hydroxyproline (16% of the residues).¹⁴ All the arabinose of the glycoprotein is linked to the polypeptide chain through the hydroxyproline residues, and the linkage is alkali-stable.³³ The high degree of allergenic cross-reactivity between Par h 1 and potato lectin and their

periodate-sensitive IgE binding (Figs. 4 and 5) further support the presence of hydroxyproline-arabinose linkages in Par h 1. It is possible that these glycopeptide structures constitute the major cross-reactive allergenic epitope(s) of Par h 1. However, the clinical significance of the cross-reactive carbohydrate-containing determinants on Par h 1 and the potato lectin is doubtful because *Parthenium*-sensitive patients do not complain of adverse symptoms on ingestion of potatoes. An explanation for this lack of symptoms of allergy to the ingested potato lectin could be its digestion or degradation in the gastrointestinal tract, such that the protein is not available to the sensitized mast cells of the gut mucosa. In this context, Bircher et al.³⁴ found a high prevalence of IgE to some fruits and vegetables in patients allergic to pollens, including patients who did not report symptoms after the intake of such foods. Hence, the presence of specific IgE to plant foods in pollen-sensitive individuals does not necessarily imply clinical disease. Therefore it seems that like tropomyosin^{15, 35} and profilin,³⁶ the ubiquitous cell wall protein extensin has certain determinants that can participate in widespread allergenic cross-reactivity. Delineation of these cross-reacting epitopes on Par h 1 could lead to the development of better strategies for the recognition and treatment of certain pollen allergies.

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