

Purification and Characterization of *Par o I*, Major Allergen of *Parietaria officinalis* Pollen

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Abstract. *Par o I*, a major allergen of *Parietaria officinalis*, was purified from the pollen extract. The purified allergen was obtained by ultrafiltration, Sephadex gel filtration and DE-52 ion exchange chromatography: the purified preparation yields a single band in polyacrylamide gel isoelectric focusing (PAG-IEF), sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting, a single immunoprecipitation arc in crossed immunoelectrophoresis (CIE) and crossed radioimmunoelectrophoresis (CRIE) and a single peak in size exclusion high-performance liquid chromatography (HPLC). *Par o I* is a glycoprotein with a protein to carbohydrate ratio of 100:21. The molecular weight, determined by SDS-PAGE, Sephadex G-50 gel filtration and size exclusion HPLC, varied between 13.5 and 14.5 kDa according to the method employed. The isoelectric point was 4.6. The amino acid composition and the sequence of the first twelve N-terminal residues were determined. The allergenicity was assayed in vivo and in vitro. 29/29 *Parietaria*-allergic patients were skin positive to *Par o I* and possessed high level of specific serum IgE antibody as it determined by radioallergosorbent test (RAST). *Par o I* contained dominant epitopes for human IgE as inhibited to 85% the pollen extract RAST performed with a pool of sera of allergic patients. The RAST inhibitory activity was not abolished by deglycosylation.

Introduction

Over the last 10 years several research teams have purified allergens which are relevant in human pathology. In Southern Europe and in the Asian and African countries bordering the Mediterranean, *Parietaria*, a plant of the family of Urticaceae, is the major cause of atopic respiratory allergy. The pollen of this weed, which grows in rural and urban environments, contains powerful allergens and in some areas it may account for up to 80% of the pollinosis [1]. From the clinical point of view, *Parietaria judaica* and *Parietaria officinalis* are the most relevant species.

The areas of diffusion of the two species are partially overlapping: *P. officinalis* prevails in Northern Italy, central France, central and Eastern Europe, *P. judaica* in the coastal areas of Spain, Southern France, Italy, Yugoslavia, Greece, Mediterranean Asia and Northern Africa [2].

The allergenic composition of the pollen extracts of these species has been investigated: both contain several allergens [3–12] and are cross-reactive [13, 14]. The major allergen of *P. judaica*, *Par j I*, has been purified by different groups: the reported molecular weight varies between 10 and 26 kDa [15–18]. Preliminary observations on the composition of the pollen

extract of *P. officinalis* and on the characterization of allergens have been reported by our group [19–22]. We describe here the purification of a major allergen, a glycoprotein with a molecular weight of 13.5–14.5 kDa.

Methods

Extraction of Pollen Allergens

P. officinalis pollen was supplied by Allergon, Engelholm, Sweden (purity 75%). The extraction procedure was performed using 1:30 w/v of pollen to 0.125 mol/l NH_4HCO_3 (pH 8.7), containing 0.05% w/v NaN_3 and 5.7×10^{-4} mol/l phenyl-methyl-sulphonyl fluorid (PMSF) to prevent proteolysis [21]. The extraction was allowed to proceed at 4°C under gentle stirring; after 4 h the suspension was centrifuged at 1,000 g for 20 min. The lyophilized supernatant (*Parietaria officinalis* PO whole extract) accounted for 23% of the dry weight of the pollen; from 1 g pollen 25.5 mg of protein was recovered.

Human Sera

The reaginic serum pool consisted of equal volumes of sera collected from 40 informed volunteers; the criteria of selection of the patients were unequivocal clinical history of allergy to *Parietaria*, skin positivity to *P. officinalis* pollen extract, high level of anti-*P. officinalis* serum antibodies (at least 25% of added cpm bound in PO-radioallergosorbent test, RAST). None of the selected serum donors had received hyposensitization therapy. The nonreaginic serum pool (used as negative control) was obtained by mixing equal volumes of sera from 10 informed volunteers without clinical allergy, skin negative to PO whole extract and with undetectable anti-PO whole extract serum antibody (less than 5% added cpm bound in PO-RAST). Both pools were stored at –20°C in aliquots of 0.5 ml until used.

Rabbit Antisera

Rabbit antisera against PO whole extract were raised in 4 rabbits. Each animal received 6 injections at intervals of 2 weeks; each dose consisted of 100 µl of antigen solution (2 mg/ml in saline) emulsified with equal volume of incomplete (first injection) or complete (following injections) Freund's adjuvant. The injections were performed intradermally in the intrascapular region distributing each dose in three sites. On the 7th day after the last injection the animals were bled; the sera were pooled and stored in aliquots of 1 ml at –20°C.

Immuno-electrophoretic Techniques

Crossed immuno-electrophoresis (CIE) and crossed radioimmuno-electrophoresis (CRIE) were essentially performed as described by Lowenstein [23]: electrophoresis in the first direction was performed for 45 min at 8.6 V/cm and in the second direction for 14 h at 2.8 V/cm in gels containing rabbit anti-PO whole extract antiserum (15 µl/cm²). In CRIE the immunoplate was incubated with 5.5 ml of the reaginic serum pool diluted 1:10 with phosphate-buffered saline (PBS) pH 7.2 and IgE binding was revealed by incubation with ¹²⁵I anti-human IgE (6.8 µCi/µg, Phadebas PRIST Pharmacia Diagnostic, Uppsala, Sweden) preadjusted to 2.0×10^5 cpm/ml in PBS pH 7.2 containing 0.3% w/v bovine serum albumin

(BSA) and 0.1% w/v ethylenediaminetetraacetic acid (EDTA). Fuji NIF X-ray films were exposed, with intensifying screens, to the immunoplates for 5 days at –80°C.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli [24]. The samples and the molecular weight standards were denaturated and reduced by boiling in 0.35 mol/l Tris-HCl (pH 6.8) containing 6% w/v SDS, 10% v/v 2-mercaptoethanol and 20% v/v glycerol, and then electrophoresed for 5 h at 24 mA. The separating gel contained a gradient (10–15%) of polyacrylamide in 225 mmol/l Tris-HCl (pH 8.8) with 0.4% w/v SDS. The proteins were stained in 0.1% w/v Coomassie brilliant blue R-250 dissolved in 40% methanol, 10% acetic acid.

Immunoblotting

The proteins separated by SDS-PAGE were transferred into nitrocellulose (BA83 Schleicher Schull) sheets as described by Towbin et al. [25]. Transfer was performed in Tris-glycine buffer pH 8.3 containing 20% v/v methanol for 14 h at 20 mA. Following transfer, the nitrocellulose sheets were soaked for 1 h at 37°C in 50 mmol/l Tris buffer pH 8.0 containing 3% w/v BSA, 0.3% v/v Tween 20, 0.9% w/v NaCl and 0.05% w/v NaN_3 and then incubated at 4°C with the human sera diluted 1:5 in 50 mmol/l Tris-HCl pH 8, containing 150 mmol/l NaCl, 0.05% w/v NaN_3 , 0.3% v/v Tween 20. After 16 h the sheets were washed and incubated for 16 h at 4°C with ¹²⁵I anti-human IgE (6.8 µCi/µg Phadebas PRIST, Pharmacia Diagnostic, Uppsala, Sweden) preadjusted to 80×10^4 cpm/ml. Fuji NIF X-ray films were exposed, with intensifying screens, to the nitrocellulose sheets for 1 week at –80°C.

Polyacrylamide Gel-Isoelectric Focusing

Isoelectric focusing (IEF) was performed, according to Wrigley [26], in 7.5% polyacrylamide gel-containing Bio-Lyte ampholytes (Bio-Rad Laboratories, Richmond, Ca., USA) in the pH range of 3–10. Standard proteins of known isoelectric point were used as markers (IEF standards, Bio-Rad Laboratories, Richmond, Ca., USA). Samples of 20 µl were applied and focused for 14 h at 2°C and 50 V/cm. The gels were fixed and stained with Coomassie brilliant blue according to Reisner [27].

Radioallergosorbent Test

The test was performed according to Ceska [28]. PO whole extract or *Par o* I were insolubilized by attachment through covalent bonds to cellulose discs punched out from Whatman 1 paper and activated by cyanogen bromide at pH 10 [29]: 2,000 activated discs were incubated with constant agitation overnight with 80 ml of PO whole extract (0.6 mg of protein/ml) or *Par o* I (0.1 mg of protein/ml) in 0.1 mol/l NaHCO_3 (pH 8.5) containing 5.7×10^{-4} mol/l PMSF and 0.05% w/v NaN_3 . To block residual active sites, the discs were reacted with a solution of ethanolamine (0.5 mol/l in 0.1 mol/l NaHCO_3). The allergosorbent discs were stored in PBS (0.9% w/v NaCl, 0.05 mol/l phosphate buffer, pH 7.4) containing 0.3% w/v BSA, 0.05% w/v NaN_3 and 0.6% v/v Tween 20. In direct RAST 50 µl of serum was incubated overnight at room temperature with each disc; after washing, 25 µl of ¹²⁵I-labelled rabbit anti-human IgE antibody (Pharmacia, Uppsala, Sweden, 6.8 µCi/µg, about 5,000 cpm/disc) was added. Bound radioactivity was measured in a Packard γ-counter. Nonreaginic serum pool bound less than 2.5%

of the added cpm. The level of serum IgE antibody specific for PO whole extract and for *Par o* I was expressed in arbitrary units (respectively, PO and *Par o* I-RAST units/ml) using as standard the reagenic serum pool (100 PO-RAST units/ml, 10 *Par o* I-RAST units/ml). A standard curve was repeated for each set of determinations.

RAST inhibition was carried out essentially as described by Gleich et al. [30]: allergosorbent discs were incubated with 50 μ l of the sample to be tested together with 12 μ l of the serum pool overnight at room temperature; bound IgE was evaluated as previously described. The inhibition percentage was calculated using the formula:

$$[1-(b-c)/(a-c)] \times 100,$$

where a = cpm bound by noninhibited reagenic serum pool, b = cpm bound by inhibited reagenic serum pool and c = cpm bound by nonreagenic serum pool. Each determination was performed in duplicate and the values reported are means.

In RAST inhibition dose response curves the inhibitors were serially 5-fold diluted; inhibition percentage was plotted against protein concentration and the protein concentration required to obtain 50% RAST inhibition (I 50%) was calculated.

Skin Prick Test

Skin prick testing was performed on the forearm of consenting adults: 20 μ l of the sample to be tested was injected with a prick test disposable needle (Hollister-Stier). Each test was repeated on the opposite arm and, after 20 min, the largest (D) and the perpendicular diameter (d) of the wheal reaction were measured; the result was given as a mean: (D + d)/2. The responses were graded from 0 to 4 according to the mean diameter of the wheal: 0–2 mm = 0, 2–3 mm = 1, 3–4 mm = 2, 4–5 mm = 3, 5–6 mm = 4. Reactions scoring at least 2 were considered positive.

Protein and Carbohydrate Determination

The protein content was determined according to the method of Bradford [31] utilizing the Bio-Rad protein assay kit and BSA as standard. The carbohydrate content was determined using the orcinosulfuric acid procedure [32].

Gel Filtration

Gel filtration through Sephadex G-75 (fine grade) was carried out in a column (bed: h = 92.5 cm, $\varnothing$ = 5.0 cm) preequilibrated with 0.125 mol/l NH_4HCO_3 containing 0.02% w/v NaN_3 . 90 mg of the retentate in 2.5 ml was applied on the column and eluted at a flow rate of 40 ml/h. 3-ml fractions were collected and absorption at 280 nm was measured.

Gel filtration through Sephadex G-50 (superfine grade) of a pool of fractions 100–135 recovered from Sephadex G-75 was carried out in a 2.6 $\times$ 90 cm column equilibrated with 0.125 mol/l NH_4HCO_3 containing 0.02% w/v NaN_3 . The sample (21.2 mg of protein in 2 ml) was eluted in the same solution at a flow rate of 24 ml/h and 3-ml fractions were collected.

Gel filtration through Sephadex G-50 (superfine grade) of the pooled fractions 78–115 recovered from ion exchange chromatography was carried out in a 0.7 $\times$ 120 cm column which had been calibrated with the following protein markers: ovalbumin, chymotrypsinogen A, myoglobin, ribonuclease, cytochrome c, myoglobin II. The sample (0.6 mg of protein in 1.2 ml) was eluted in 0.125

mol/l NH_4HCO_3 containing 0.02% w/v NaN_3 at a flow rate of 10 ml/h; 1-ml fractions were collected.

Ion Exchange Chromatography on DE-52

Ion exchange chromatography on DE-52 (Whatman Biochemicals, Maidstone) was performed in a 0.6 $\times$ 48 cm column: pooled fractions 36–48 recovered from Sephadex G-50 gel filtration were loaded on the column; 30 fractions (1 ml each) were collected eluting with 5 mmol/l Tris-HCl pH 7.8 at a flow rate of 18 ml/h. To recover the bound material two gradients of increasing NaCl concentration (0.0–0.2 and 0.2–2.0 mol/l) were run sequentially at the same flow rate.

Deglycosylation

Par o I was deglycosylated with trifluoromethanesulfonic acid for 2 h at 25 $^{\circ}\text{C}$ according to Edge et al. [33]. The deglycosylated protein was diluted with PBS and ultrafiltered through Amicon YM2 membrane.

Affinity Chromatography

Par o I (0.4 mg of protein) was chromatographed on a 0.6 $\times$ 8.5 cm column of Con A-Sepharose (Pharmacia, Uppsala, Sweden). 20 mmol/l Tris-HCl pH 7.4 containing 0.5 mol/l NaCl, 1 mmol/l Ca^{2+} , 1 mmol/l Mn^{2+} was used to recover the unbound fraction; the bound fraction was eluted with 0.5 mol/l α -methyl-D-glucoside dissolved in the same solution.

High-Performance Liquid Chromatography

Size exclusion high-performance liquid chromatography (HPLC) was performed on Waters (Millipore, Milford, Ma., USA) equipment using a Protein-Pak TM 60 column (supplied by the same manufacturer, 0.78 $\times$ 30 cm; packing material: bonded silica gel; particle size: 10 μ) which provides separation in the molecular weight range 1–20 kDa. The mobile phase was 0.1 mol/l phosphate buffer pH 7.0 pumped at a constant flow rate of 1 ml/min; approximately 1.7 μ g of *Par o* I was injected; A 280 nm was registered. The column had been calibrated with the following molecular weight markers: blue dextran (2,000 kDa), myoglobin (17.0 kDa) ribonuclease A (13.7 kDa), cytochrome c (12.3 kDa), oxytocin (1.0 kDa).

Amino Acid Composition

Par o I was hydrolyzed in 5.8 mol/l HCl in vacuo for 24 h at 110 $^{\circ}\text{C}$ and the amino acid composition was determined using an integrator connected to a Carlo Erba automatic analyzer. Cysteine was determined as cysteic acid (after performic acid oxidation of the protein). Tryptophan was determined after hydrolysis in 3 mol/l mercaptoethanesulfonic acid.

N-Terminal Amino Acid Sequence

Automatic Edman degradation was performed with an Applied Biosystem 477A Protein Sequencer: approximately 1.5 nmoles of *Par o* in 50 μ l of distilled water were injected.

Results

Characterization of PO Whole Extract

The antigenic and allergenic composition of the aqueous pollen extract of *Parietaria* was analyzed by

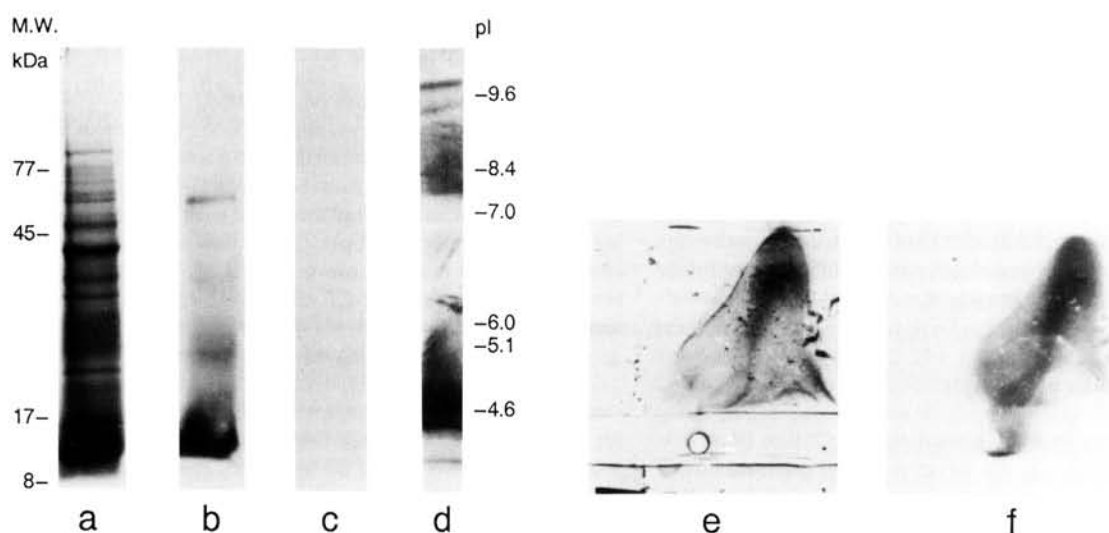


Fig. 1. Analyses of PO whole extract. **a** SDS-PAGE. **b**, **c** Immunoblotting with reagenic (**b**) and nonreagenic (**c**) serum pool. **d** PAGE-IEF. **e** CIE. **f** CRIE. Amount of protein applied: 150 μ g (**a**–**c**), 210 μ g (**d**), 145 μ g (**e**, **f**).

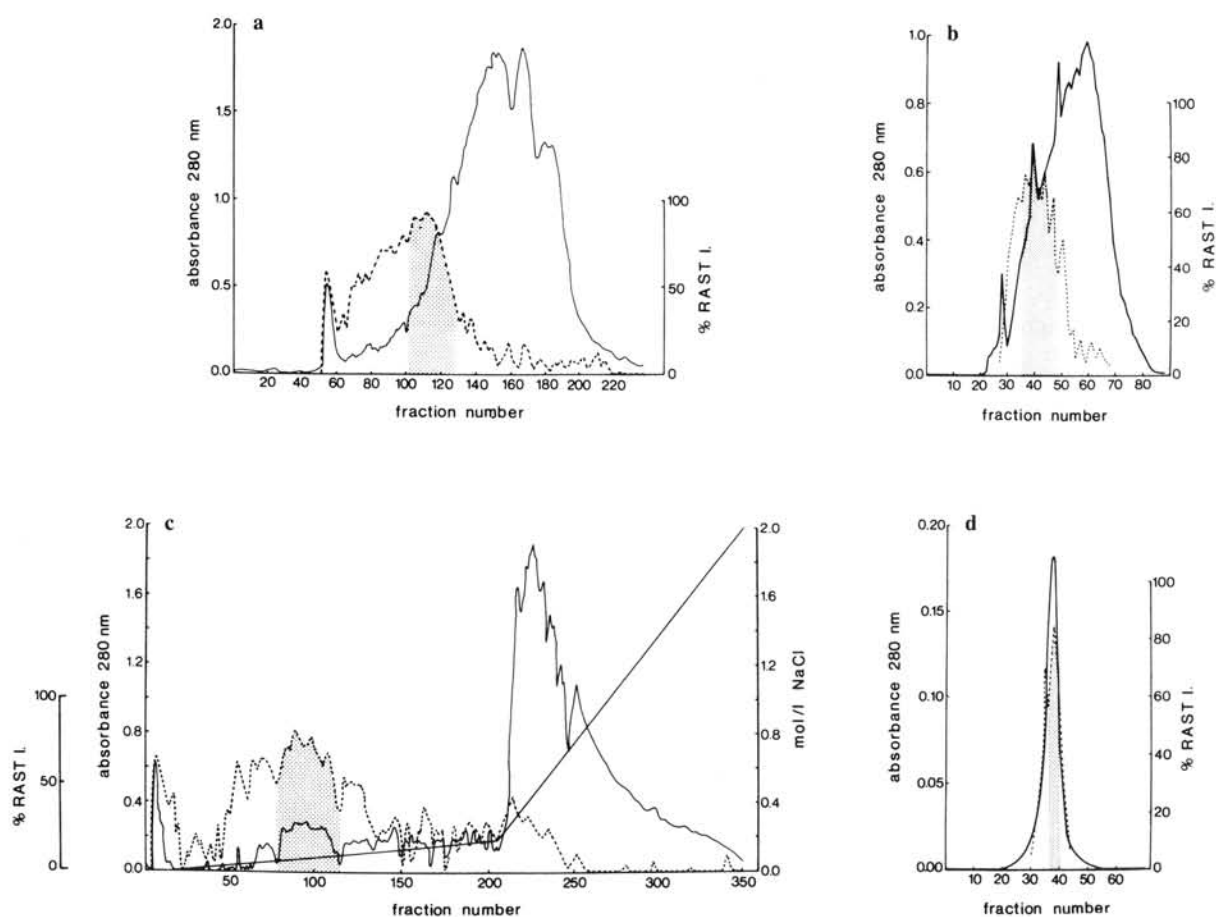


Fig. 2. Purification of *Par o I*. **a**, **b** Sephadex G-75 (**a**) and G-50 (**b**) gel filtration. **c** DE52 ion exchange chromatography. **d** Sephadex G-50 gel filtration. — = Absorbance at 280 nm; --- = % RAST inhibition (I). Shaded areas represent fractions used in each subsequent purification step.

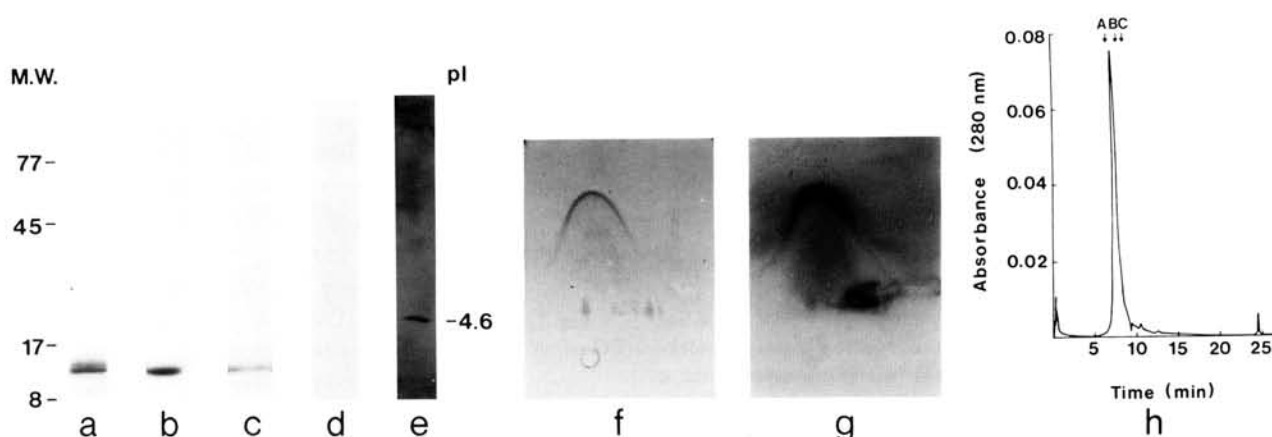


Fig. 3. Characterization of *Par o I*. **a** Pool of fractions 36–48 from Sephadex G-50 gel filtration. **b–h** *Par o I*. **b** SDS-PAGE. **c, d** Immunoblotting with reagenic (**c**) and nonreagenic (**d**) serum pool. **e** PAG-IEF. **f** CIE. **g** CRIE. **h** Size exclusion HPLC (A, B and C: elution times of myoglobin, cytochrome c and insulin, respectively). Amount of protein: 44 μ g (**a**), 30 μ g (**b–d**), 50 μ g (**e**), 25 μ g (**f, g**), 1.7 μ g (**h**).

SDS-PAGE, immunoblotting, CIE, CRIE, and PAG-IEF. PO whole extract contained, as shown by SDS-PAGE, at least 30 protein components with a molecular weight ranging between 90 and 8 kDa (fig. 1a); at least 8 components bound human IgE of the reagenic serum pool in immunoblotting (fig. 1b); no binding was observed with the nonreagenic serum pool (fig. 1c). PAG-IEF analysis revealed components focused within the pH range 4.0–10.0 (fig. 1d). 11 arcs with anodic and 2 arcs with cathodic mobility were observed when the whole extract was tested against rabbit antiserum by CIE (fig. 1e): of these 3 bound specific IgE of the reagenic serum pool in CRIE (fig. 1f).

Par o I Purification

Par o I was obtained using five steps of purification: (1) Low-molecular-weight components were discarded from PO-whole extract by ultrafiltration through YM 5 Amicon membrane (cutoff: 5 kDa): 86% of the protein and 63% of the carbohydrate were recovered in the retentate (final concentration 100 $\times$). The specific IgE-binding activity of the retentate, determined by PO-RAST inhibition, was 3.1-fold higher than that of the whole extract and 18-fold higher than that of the ultrafiltered fraction. (2) The retentate was gel-filtered through Sephadex G-75 (details in Methods) (fig. 2a); the IgE-binding activity of single fractions was assessed by RAST inhibition. The main UV absorbing peak, probably a chromophore (high absorbance at 410 nm), was associated with very low IgE-binding activity while the highest activity was re-

covered in fractions 100–135. A pool of these fractions represented 24.5% of the protein content of the retentate: the apparent molecular weight of the protein components ranged between 19.5 and 10.0 kDa as documented by SDS-PAGE. (3) Pool 100–135 was gel-filtered through Sephadex G-50 (details in Methods) (fig. 2b). The fractions associated with the highest IgE-binding activity (36–48) were pooled. This pool represented 12% of the protein content of the sample applied to the column; SDS-PAGE analysis indicated that it contained a doublet of distinct bands with the apparent molecular weight of 14.5–15.0 kDa (fig. 3a): the lower band was more abundant but both were able to bind IgE of the reagenic serum pool in immunoblotting. (4) To resolve the doublet we performed ion exchange chromatography on DE-52 eluting with an ionic strength gradient (details in Methods) (fig. 2c). Active peaks were recovered both in unbound and bound fractions; residual chromophore was eluted with 0.2 mol/l NaCl. The higher band of the doublet was recovered essentially in the unbound fraction, while the lower component was recovered in fractions 78–115 and represented 3% of the protein content of the chromatographed sample. (5) An additional step of gel filtration on Sephadex G-50 was carried out to further purify the 14.5-kDa allergen (details in Methods) (fig. 2d). The elution profile shows a single, narrow, symmetric UV-absorbing peak essentially coincident with the RAST inhibitory activity peak. A pool of fractions 37, 38 and 39 represented 60% of the protein content of the sample applied to the column and was designated as *Par o I*.

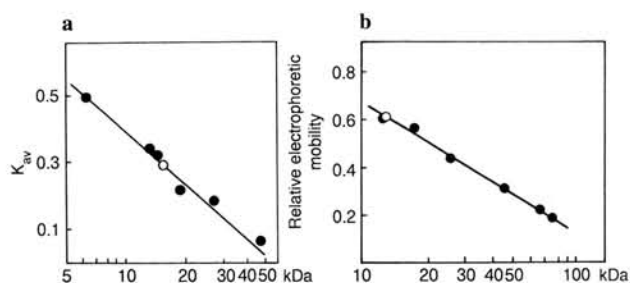


Fig. 4. Determination of the molecular weight of *Par o I*. **a** Sephadex G-50 gel filtration. **b** SDS-PAGE. Molecular weight markers were the following. **a** Myoglobin II (6.2 kDa), cytochrome c (12.3 kDa), ribonuclease A (13.7 kDa), myoglobin (17 kDa), chymotrypsinogen A (25.7 kDa), ovalbumin (45 kDa). **b** Cytochrome c, myoglobin, chymotrypsinogen A, ovalbumin, albumin (66 kDa) and ovotransferrin (76.0 kDa).

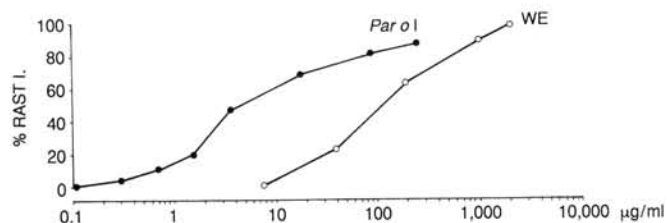


Fig. 5. RAST inhibition (I) dose response curves of *Par o I* and PO whole extract (WE) (details in Methods).

Table 1. Analysis of the amino acid composition of *Par o I*

Amino acid	mol/100 mol	Residues/molecule
Lys	7.6	8
His	1.8	2
Arg	2.6	2 or 3
Asx	7.9	8
Thr	7.4	7 or 8
Ser	6.8	7
Glx	11.8	12
Pro	6.8	7
Gly	11.7	12
Ala	7.7	8
Cys	3.8	4
Val	5.8	6
Met	1.8	2
Ile	4.0	4
Leu	6.6	7
Tyr	1.6	1 or 2
Phe	3.2	3
Trp	1.0	1
Total	99.7	101 or 104

45 μg of *Par o I* was recovered from 100 mg of pollen extract protein.

Biochemical and Immunochemical Characterization of *Par o I*

In SDS-PAGE *Par o I* migrated as a single component (fig. 3b) that bound specific IgE in immunoblotting (fig. 3c, d). *Par o I* focused as a single band in

PAG-IEF (fig. 3e); in size exclusion HPLC analysis it was eluted as a single peak that accounted for 97% of the UV absorbing material (fig. 4h). In CIE and CRIE analyses it yielded a single immunoprecipitation arc with anodic mobility (fig. 3f, g).

The glycoprotein allergen had a protein to carbohydrate ratio of approximately 100:21 (protein and carbohydrate content determined according to the Bradford and orcinol-sulfuric method, respectively). The presence of α -D-mannopyranosyl or α -D-glucopyranosyl end groups was excluded by the absence of concanavalin A (Con A) binding in Con A-Sepharose affinity chromatography. The carbohydrate moiety did not contain major epitopes for IgE antibodies as the deglycosylated (by trifluoromethanesulfonic acid) protein retained 90% of the original RAST inhibition activity.

The extinction coefficient E (1%, 1 cm), determined at 280 nm, was 13.2.

The molecular weight varied between 13.5 and 14.5 kDa according to the method employed (fig. 4): in reducing conditions, determined by SDS-PAGE, it was 14 kDa; in native conditions, by size exclusion HPLC and Sephadex G-50 gel filtration it was 13.5 and 14.5 kDa, respectively.

The protein moiety of *Par o I* contained all the naturally occurring amino acids including 4 residues of cysteine (table 1); the molecular weight of the peptide moiety, calculated on the basis of 4 isoleucine residues/molecule of protein, ranged between 12,443 and 12,917. Twelve N-terminal residues were sequenced by automatic Edman degradation:

A T G K (V or T) V Q (G or D) (A or I) M P P.

The molar yield of the first residue (alanine) was 75%.

Par o I gave maximum inhibition values close to 85% in the RAST inhibition dose response curve with the reaginic serum pool (fig. 5). The IgE-binding specific activity ($I_{50\%} = 4.6 \mu\text{g}$ of protein/ml) of the purified allergen was 24-fold higher than that of PO whole extract.

Allergenicity

The allergenicity of *Par o I* was assayed in vitro and in vivo. 29 patients with clinical allergy to *Parietaria* documented by clinical history and by positive (as defined under Methods) skin prick test to the PO-whole extract were analyzed for cutaneous reactivity to *Par o I* and for *Par o I* specific serum IgE antibody.

The cutaneous reactivity was determined by skin prick test using the purified allergen at a concentration of $40 \mu\text{g}$ of protein/ml. All patients were positive: 23 of them scoring 4, 4 scoring 3 (positivity was graded as described in Methods).

Par o I and PO whole extract specific serum IgE antibody levels were evaluated by RAST and expressed as units per milliliter (details in Methods): 16/29 sera contained more than 10 units/ml and 6/29 between 2 and 10 units/ml of *Par o I*-specific IgE antibody. Figure 6 shows a comparison between PO and *Par o I*-RAST values (units/ml): a positive correlation was observed ($r = 0.36$; $p < 0.03$).

Discussion

The homogeneity of *Par o I* was assessed by SDS-PAGE, PAG-IEF, CIE and HPLC. In SDS-PAGE the *Par o I* band appeared single and clear and, when transferred into nitrocellulose, it bound IgE of the reaginic serum pool (fig. 3b–d). In CIE and CRIE *Par o I* yield a single and rather asymmetrical immunoprecipitation arc (fig. 3f, g).

Amino acid analysis yields as most representative residues Glu (or Gln), Gly and Lys. Four Cys and seven Pro residues were present (table 1).

The sequence of twelve N-terminal amino acid residues has been determined. At three positions it was impossible to assign a single amino-acidic residue: a similar amount of two different residues was recovered in the 5th, 8th and 9th cycle of the automatic

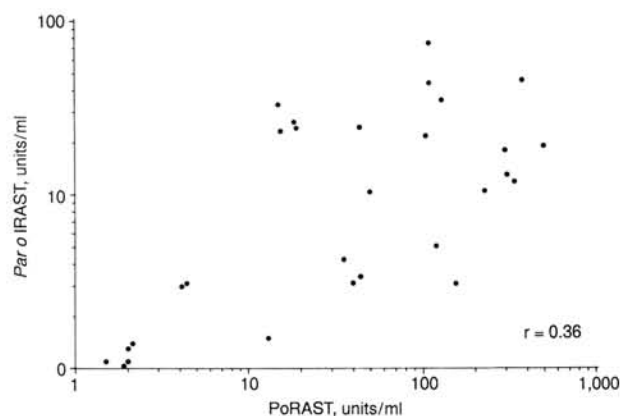


Fig. 6. Comparison of the level of IgE antibody to *Par o I* and PO whole extract in the sera of 29 *Parietaria*-allergic subjects.

degradation; this observation may be explained by a limited heterogeneity of *Par o I*. Purified allergen preparations have often been reported to consist of several molecular species, indistinguishable by most physicochemical parameters (isoforms). Considering the outbreeding of the plant population it is conceivable that these represent isotypic, allelic or species variants: further biochemical characterization and studies at the DNA level may clarify this point.

Par o I has dominant epitopes for human IgE antibody as documented by its high inhibitory activity (85%) in the PO-RAST performed with the reaginic serum pool (fig. 5). Epidemiologically, it is a major allergen: 29/29 *Parietaria* allergic subjects studied were skin-positive to the purified allergen. In addition the level of anti *Par o I* IgE antibody was significantly correlated to the level of anti-PO whole extract IgE antibody.

The dominant epitopes are contained in the protein moiety of *Par o I* as the deglycosylation did not abolish RAST inhibitory activity: the drastic reaction conditions may explain the limited loss of activity observed (10%).

Our results do not exclude the presence in the whole extract of additional major allergens sharing dominant epitopes with *Par o I*, such as the higher molecular weight component of the doublet (fig. 3a). The structural relationship of *Par o I* with this and other components of the pollen extract remains to be investigated.

The relationship of *Par o I* with the major allergen of *P. judaica*, *Par j I*, is an open question as the two allergens have not been assayed for cross-reactivity.

However, most parameters, molecular weight [18], electrophoretic mobility in CIE [17], isoelectric point [15], presence of carbohydrate [34], are similar. The amino acid composition [17, 18] and the sequence of twelve N-terminal residues [Polo, pers. commun.] show extensive similarity.

Par o I and *Par j I* have the lowest molecular weight among major pollen allergens. This feature, that is advantageous to achieve a full characterization of the physicochemical structure, singles them out as good candidates to investigate the relationship between the structure of allergens and their function in the immune system.

The results of these studies may be important for the standardization of current reagents for immunotherapy and to promote advances in the technology of vaccines.

Acknowledgement

Our thanks are due to Dr. G. Sacerdoti for providing human sera and performing skin tests.

Parts of this work were supported by grants from CNR, 'Progetto speciale Peptidi' and CNR Target Project Biotechnology and Bio-Instrumentation.

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Received: August 15, 1990

Accepted after revision: April 19, 1991

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