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IDENTIFICATION OF IgE BINDING POLYPEPTIDES CROSS-REACTIVE WITH THE *PARIETARIA* *JUDAICA* MAIN ALLERGENIC POLYPEPTIDE

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Abstract—The major allergen of the pollen of *Parietaria judaica* was characterized using an anti-allergen MAb AC/15.1. The antibody was able to immunoadsorb four different polypeptides (10,000, 20,000, 30,000 and 40,000 mol. wt) from the pollen proteins radioiodinated by the Bolton-Hunter's reagent. The four polypeptides have been shown not to be covalently linked, except for the 10,000 mol. wt polypeptide (Pj10), which appeared to form Pj10 dimers under non-reducing conditions. All of them contained the antigenic epitope defined by the monoclonal antibody and demonstrated human IgE binding ability. The structural relationship of these polypeptides in the native allergen is discussed.

INTRODUCTION

The pollen of *Parietaria judaica* is responsible for a high percentage of allergic diseases in the South and West of Europe (Errigo *et al.*, 1982).

IgE-binding antigens (allergens) are among those components whose purification is impaired by the complex nature of the sources they come from, usually ill-defined materials (Krilis *et al.*, 1981). In order to facilitate this purification, MAbs† directed to allergens from *Lolium perenne* pollen (Smart *et al.*, 1983; Ekramoddoullah *et al.*, 1984) and *Dermatophagoides* mites (Chapman *et al.*, 1984; Ley *et al.*, 1985) have been already produced.

In a previous paper (Corbi *et al.*, 1985a) three MAbs were described (AC/1.1, AC/7.1, AC/15.1) to the major allergen of *P. judaica* pollen. The purification of the major allergen was carried out by monoclonal antibody-affinity chromatography, and it was found to contain the main IgE-binding polypeptide detected by immunoblotting (Corbi and Carreira, 1984). Although several denatured components displayed IgE-binding ability, RAST-inhibition studies revealed the purified allergen was responsible for, at least, 80% of the total IgE binding capacity of the pollen. For this reason, the structures recognized by anti-allergen MAb have been subjected to further study.

In the present paper evidence is shown suggesting the major allergen from *P. judaica* pollen is formed by several polypeptides which are antigenically cross-reactive and exhibit IgE-binding ability.

MATERIALS AND METHODS

Monoclonal antibodies

AC/15.1 anti-*P. judaica* pollen major allergen MAb was produced in our lab (Corbi *et al.*, 1985a). This MAb as well as the anti-human IgE HE2/1 MAb (Sánchez-Madrid *et al.*, 1984) were purified on protein A-Sepharose (Ey *et al.*, 1978) and radiolabeled with ¹²⁵I-Na using chloroglycoluril (Fraker and Speck, 1978).

Affinity chromatography

P. judaica pollen major allergen was purified on an AC/15.1 MAb-Sepharose 5 ml column. The immunosorbent was prepared by coupling the purified MAb to CNBr-activated Sepharose according to the method of Cuatrecasas (1970). One milliliter (37 mg) of pollen extract (Corbi and Carreira, 1984) was passed through the column which had been previously washed with 3 M KSCN in PBS. After extensive washing with PBS the bound allergen was eluted with 3 M KSCN in the same buffer, dialyzed against PBS, distilled water and lyophilized. Purity was checked by SDS-PAGE.

Radiolabeling and immunoadsorption

Major allergen and whole pollen extract were radioiodinated using the Bolton-Hunter's reagent (Bolton and Hunter, 1973). A preclearing step was performed with normal rabbit IgG-Sepharose to eliminate components with non-specific binding to the antibody-Sepharose complex. Then, either ¹²⁵I-labeled pollen proteins or ¹²⁵I-major allergen were

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†Abbreviations: MAb(s), monoclonal antibody(ies); CNBr, cyanogen bromide; KSCN, potassium sulfocyanate; PBS, phosphate-buffered saline; SDS-PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate; mol. wt, molecular weight; Pj10, 10,000 mol. wt allergenic polypeptide; BSA, bovine serum albumin.

incubated by shaking for 1 hr with AC/15.1 MAb-Sephacrose. After washing three times with washing buffer (0.1% bovine hemoglobin and 0.1% Triton X-100 in PBS), once with PBS and once more with 50 mM Tris-HCl, pH 6.8, samples were incubated with Laemmli's sample buffer for 10 min at 100°C and subjected to SDS-PAGE (Laemmli, 1970) under reducing conditions, unless otherwise indicated. Finally, gels were dried and autoradiographed with intensifying screens (Laskey and Mills, 1977).

Alternatively, labeled pollen proteins were immunoadsorbed with HE2/1 MAb-Sephacrose, previously incubated with a *P. judaica* sensitive serum pool, following the same procedure above described (Corbi *et al.*, 1985a).

Two-dimensional electrophoresis

Samples were loaded on 14% polyacrylamide gels containing SDS, under the standard conditions. After completion of the first dimension non-reducing electrophoresis, strips were cut out of the gel, equilibrated in Laemmli's sample buffer containing 5% β -mercaptoethanol for 30 min and placed on top of a 14% polyacrylamide gel for the second-dimension electrophoresis.

Two-dimensional electrophoresis with reducing agent in both dimensions was carried out when indicated, following the same procedure, but with increasing concns of SDS (0.1–5%) in the second run.

Preparative SDS-PAGE

To separate the different polypeptides of the ^{125}I -major allergen, a 10^7 cpm sample was subjected to SDS-PAGE under reducing conditions. Once Bromophenol Blue reached the end of the gel, polypeptides were located by gamma-counting. Polypeptide-containing strips were excised, homogenized and incubated with 2 ml of 50 mM Tris-HCl, pH 6.8, 0.1% SDS, for 16 hr at room temp. The homogenates were then centrifuged and filtered to eliminate pieces of polyacrylamide gel and the eluted polypeptides were analyzed by SDS-PAGE and immunoadsorbed as described above.

RESULTS

In a previous paper (Corbi *et al.*, 1985a) three MAbs have been described which specifically recognized the *P. judaica* pollen major allergen and they were shown by sequential immunoadsorption to be directed to epitope(s) located on the same structure (Pj10 polypeptide). On the other hand, cross-competition experiments demonstrated that all of them recognized the same or closely related epitopes on the allergen molecule with very similar binding capacity (results not shown). Consequently, only AC/15.1 MAb was used to study the molecular characteristics of the major allergen of *P. judaica* throughout the present work.

Pollen components recognized by anti-allergen monoclonal antibodies

To determine whether AC/15.1 MAb was able to react with some other previously undetected pollen components, the extract was radiolabeled with the Bolton-Hunter's reagent and immunoadsorbed with AC/15.1 MAb-Sephacrose. Two polypeptides of 20,000 and 30,000 mol. wt were co-adsorbed with the Pj10 polypeptide (Fig. 1). In addition, over-exposure of the film revealed a 40,000 mol. wt band. Previous experiments in which pollen proteins were radioiodinated using chloroglycoluril (Corbi *et al.*, 1985a) showed only a slight radiostaining of the first two bands, suggesting that radiolabeling on amino groups allows a better detection of the antigens recognized by the anti-Pj10 MAbs.

As Pj10 polypeptide has been reported to be linked to other pollen components (Corbi and Carreira, 1984), a two-dimensional PAGE was carried out to study whether the 20,000, 30,000 and 40,000 mol. wt structures were individual polypeptides or were formed by aggregated Pj10 units not fully dissociated.

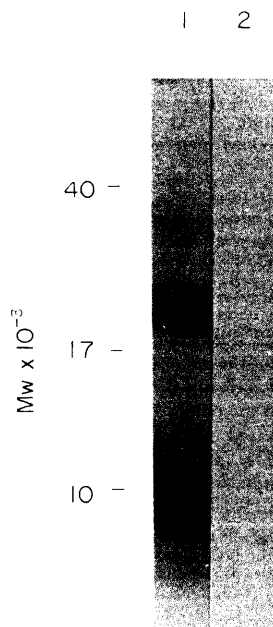


Fig. 1. Immunoadsorption of the *P. judaica* pollen major allergen. Bolton-Hunter's radiolabeled pollen proteins were incubated with 25 μl of AC/15.1 MAb (lane 1) or HE2/1 MAb-Sephacrose (lane 2). After shaking for 1 hr at 4°C, the beads were washed and boiled for 10 min in Laemmli's sample buffer. Reduced samples were subjected to SDS-14% PAGE and autoradiographed with intensifying screens. Molecular wt was determined according to the position of catalase (60,000 mol. wt), aldolase (40,000 mol. wt), carbonic anhydrase (29,000 mol. wt), myoglobin (17,000 mol. wt) and cytochrome c (12,500 mol. wt).

Once the polypeptides were separated in the first dimension (0.1% SDS), the second run was achieved with increasing amounts of anionic (SDS) detergent (0.1–5%). In all cases (Fig. 2 illustrates only 2% SDS) none of the higher mol. wt polypeptides yielded lower mol. wt structures, suggesting the 20,000, 30,000 and 40,000 mol. wt polypeptides were not formed by the aggregation of Pj10 units, but were individual structures.

The co-adsorption of these polypeptides indicated they could be linked to each other, giving rise to a high mol. wt allergen under non-denaturing conditions. As Pj10 has been reported to be attached to other structures by disulphide bonds (Corbi and Carreira, 1984), the presence of intrachain bridges was tested by two-dimensional electrophoresis (Fig. 3). After the first dimension, a broad band appeared covering a range of mol. wt from 30,000 to 20,000, but it was resolved into the 30,000, 20,000 and 10,000 mol. wt bands in the second dimension (with β -mercaptoethanol). The presence of the 40,000 mol. wt polypeptide could not be clearly shown in this experiment. The position of each one of the bands demonstrated they were not attached by disulphide bridges. However, Pj10 polypeptide was shown to contain interchain bonds, originating Pj10 dimers

under non-reducing conditions. On the other hand, the 20,000 and 30,000 mol. wt polypeptides seemed to contain intrachain disulphide bridges, for their mobilities were decreased in the presence of β -mercaptoethanol. These two facts can cause the close migration of the three structures (Pj10 dimers, 20,000 and 30,000 mol. wt) under non-reducing conditions, although they were not linked by disulphide linkages.

Distribution of the antigenic and allergenic determinants

Considering that the polypeptides adsorbed by anti-*P. judaica* pollen major allergen MAb were not covalently linked, the co-adsorption observed might be explained by either non-covalent bonds or presence of the AC/15.1 MAb-defined epitope in all structures or both. To study which possibility was correct, ^{125}I -labeled major allergen was dissolved in a number of buffers with different pH and ionic strength. After incubation, the allergen was immunoadsorbed with AC/15.1 MAb-Sephacrose (Fig. 4). In no case could a unique polypeptide be retained, even after treatment with pH 3 or pH 11 buffer. It suggests the antigenic epitope is located on all the immunoadsorbed structures, that is, on Pj10, 20,000,

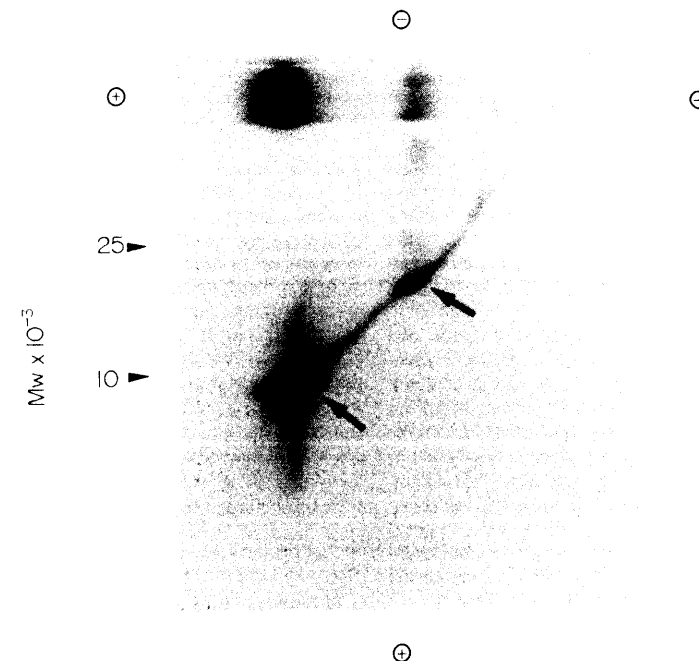


Fig. 2. Two-dimensional electrophoresis of the immunoadsorbed major allergen in presence of β -mercaptoethanol. After the first dimension (0.1% SDS; 14% acrylamide, from right to left) a gel strip was cut out and subjected to a second electrophoresis (14% acrylamide from top to bottom) with higher concns (2%) of SDS in the sample buffer and gel. The arrows indicate the position of Pj10 and 20,000 mol. wt polypeptides. Molecular wt was determined as in Fig. 1.

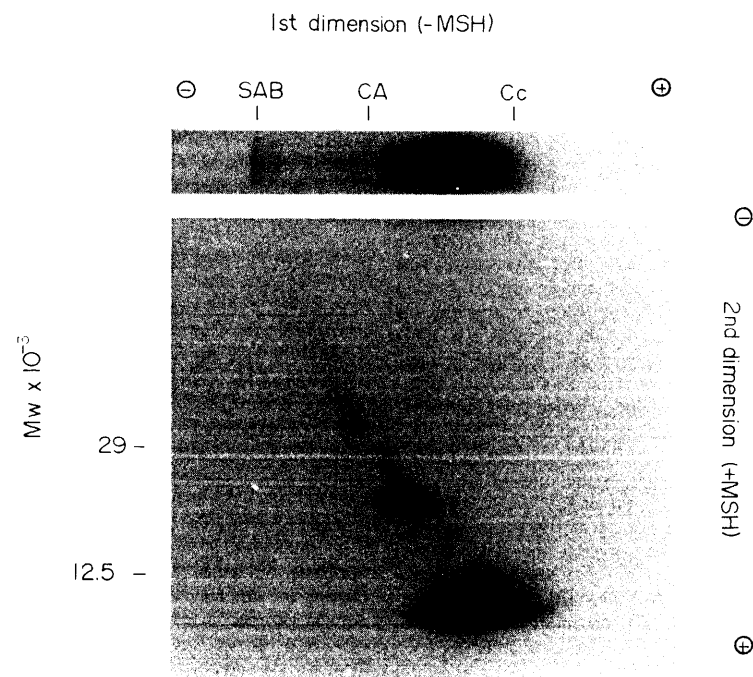


Fig. 3. Two-dimensional (non-reducing/reducing) electrophoresis of the immunoadsorbed major allergen. The procedure was as described in Fig. 2, except for the lack of reducing agent in the first dimension, and a similar concn of SDS (0.1%) in both gels. Molecular markers were bovine serum albumin (BSA: 66,000 mol. wt), carbonic anhydrase (CA: 29,000 mol. wt) and cytochrome *c* (Cc: 12,500 mol. wt).

30,000 and 40,000 mol. wt polypeptides. Furthermore, when the immunoadsorption was carried out with SDS in the incubation buffer (0.1–0.5%), all the components were again bound by the antibody, even in the presence of 0.5% SDS (Fig. 4). This feature appeared to confirm the ubiquitous presence of the antigenic epitope.

A further verification of this hypothesis was obtained by immunoadsorption of the electrophoretically separated Pj10, 20,000 and 30,000 mol. wt polypeptides (Fig. 5). Once they were eluted out of the gel and immunoadsorbed with AC/15.1 MAb–Sepharose, retained molecules were analyzed by SDS–PAGE. Again, the AC/15.1 MAb was able to bind the three polypeptides [Fig. 5(A), (B)] indicating the presence of the AC/15.1 MAb-defined epitope on those structures. The small amount of the radioiodinated 40,000 mol. wt polypeptide on the gel did not permit us its further immunoadsorption.

In addition, based on a previous identification of two allergenic polypeptides of 20,000 and 30,000 mol. wt by immunoblotting (Corbi and Carreira, 1984), the IgE-binding ability of the separated polypeptides was studied. The electrophoretically isolated components were immunoadsorbed on a HE2/1 MAb (anti-human IgE)–Sepharose column which had been

previously incubated with a *P. judaica* sensitive patients serum pool [Fig. 5(C)]. Pj10, 20,000 and 30,000 mol. wt polypeptides were retained by the column, demonstrating they contain IgE-binding epitopes in addition to the AC/15.1 MAb-binding determinant(s). This fact was later confirmed by bioaffinity chromatography of ^{125} I-labeled major allergen on specific IgE–HE2/1 MAb–Sepharose, where all the polypeptides (including the 40,000 mol. wt one) could be observed (Fig. 6).

Taking into account all the above results, it can be concluded that the *P. judaica* major allergen, which is immunoadsorbed by AC/15.1 MAb, consists of, at least, four antigenically cross-reactive polypeptides with IgE-binding ability.

DISCUSSION

In the present paper four polypeptides have been described which are co-adsorbed with the main IgE-binding structure of *P. judaica* pollen by anti-*P. judaica* pollen major allergen AC/15.1 MAb. They had not been so clearly observed on previous experiments after pollen proteins being radiolabeled either with Chloramine-T or Iodogen methods. The specific radioactivity of the four polypeptides (Pj10, 20,000,

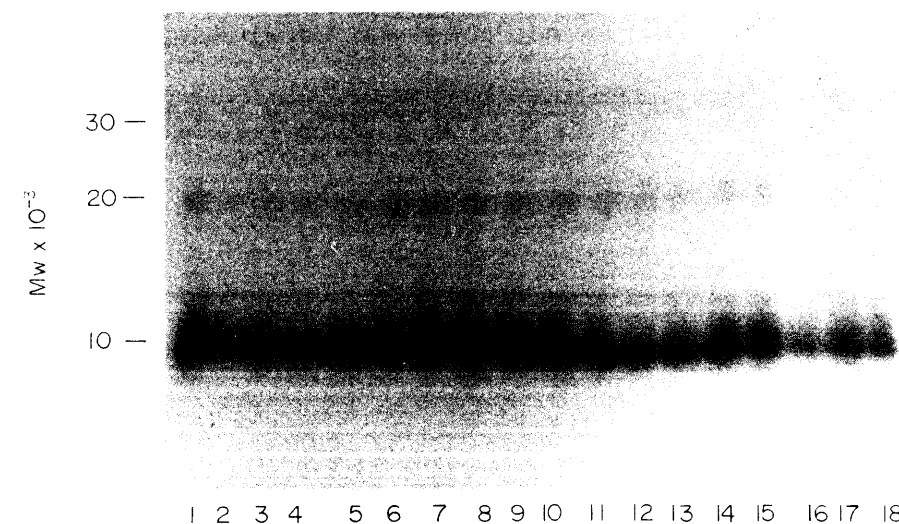


Fig. 4. Immunoadsorption of the major allergen after different treatments (lanes 1–10) or in the presence of increasing concns of detergents (lanes 11–18). The ^{125} I major-allergen was incubated for 1 hr with 0.2 M glycine–HCl, pH 2.4 (lane 1), 0.1 M citrate buffer, pH 3.4 (lane 2), 0.5 M NaCl in 0.1 M acetate buffer, pH 4.0 (lane 3), 0.1 M citrate buffer, pH 5.0 (lane 4), 0.1% Triton X-100 in 0.05 M PBS (lane 5), 0.5 M NaCl in 0.1 M NaCO₃H, pH 8.0 (lane 6), 1 M NaCl, 0.1% Triton X-100 in 0.02 M glycine, pH 7.0 (lane 7), 0.15 M NaCl in 0.02 M glycine, pH 11 (lane 8), 1 M NaCl, pH 7.0 (lane 9) and 0.05 M Tris–HCl, pH 8.0 (lane 10). Then pH was equilibrated to 7.4, and the immunoadsorption proceeded as usual. Simultaneously, immunoadsorptions were carried out with increasing amounts of detergent in the incubation buffer: 0.1% Triton X-100 (lane 11), 0.2% Triton X-100 (lane 12), 0.4% Triton X-100 (lane 13), 0.8% Triton X-100 (lane 14), 1% Triton X-100 (lane 15), 2% Triton X-100 (lane 16), 0.1% Triton X-100, 0.1% SDS (lane 17) and 0.1% Triton X-100, 0.5% SDS (lane 18). After incubations, all of them were washed, eluted and analyzed by SDS–14% PAGE and autoradiography. Molecular wt was determined according to the position of heavy and light chain of the solid-phase-bound AC/15.1 MAb.

30,000, 40,000 mol. wt) was sensibly increased with Bolton–Hunter's reagent, pointing out the fact that amino groups should be more easily available for radioiodination or more abundant than aromatic residues. The usefulness of this method for labeling polypeptides with a poor number of tyrosines has been reported (Ciccimarra *et al.*, 1975). Furthermore, the Chloramine-T method and Iodogen procedure caused a higher proteolytic breakdown of the radio-labeled allergen, a fact already demonstrated (Opresko *et al.*, 1980). Even so, a great amount of purified allergen (labeled or non-labeled) was required for clear detection of the 20,000, 30,000 and 40,000 mol. wt polypeptides, both by autoradiography or Coomassie Blue staining (Corbi *et al.*, 1985a). It leads to the conclusion that Pj10 is the main component of the allergen, whereas the rest of polypeptides are minor components.

Two-dimensional electrophoresis (non-reducing/reducing) has proved these polypeptides are not covalently linked, except for the case of Pj10 dimers. Moreover, immunoadsorption of the ^{125} I-labeled major allergen after dissociating treatments (pH 3, pH 11, high ionic strength, etc.) showed all the polypeptides were retained by AC/15.1 MAb, sug-

gesting all of them contained the AC/15.1 MAb-defined epitope. This fact was later confirmed for the electrophoretically purified Pj10, 20,000 and 30,000 mol. wt polypeptides by means of immunoadsorption on AC/15.1 MAb–Sepharose, what demonstrated an antigenic cross-reactivity among them. In addition, the four structures were individually recognized by *P. judaica* specific IgE, that is, they possess IgE-binding ability.

The presence of common antigenic determinants in allergens from a certain source has already been reported. Lowenstein *et al.* (1981) have found common epitopes on AgE, AgK, Ags 11 and 27a allergens from ragweed pollen. Similar results have been obtained for AgE–AgK and Ra 4 from ragweed pollen (Griffiths and Brunet, 1971) and Group I, II and III ryegrass pollen allergens (Hull *et al.*, 1979).

The similarity of these data with those obtained in *P. judaica* pollen appears to suggest that common antigenic epitopes can be found in a number of different proteins from an allergen source.

Considering the antigenic cross-reactivity among Pj10, 20,000, 30,000 and 40,000 mol. wt polypeptides, as well as the shared IgE-binding ability, the possibility was tested that AC/15.1 MAb was directed to

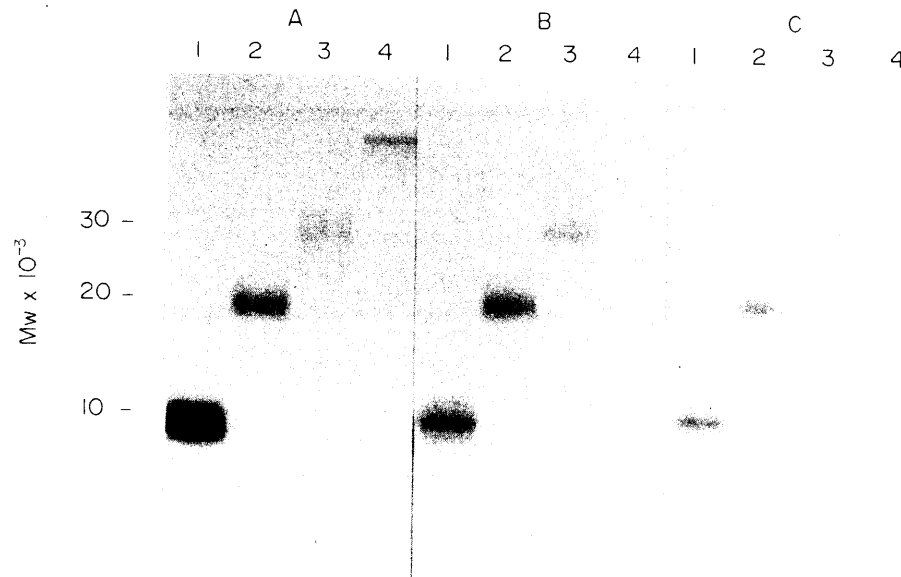


Fig. 5. Immunoadsorption of electrophoretically separated Pj10, 20,000 and 30,000 mol. wt polypeptides. The radioiodinated major allergen was subjected to preparative SDS PAGE and the radiolabeled polypeptides located by gamma-counting strips of the gel. They were eluted out of the gel with 50 mM Tris-HCl, pH 6.8, 0.1% SDS for 16 hr and tested for extraction by SDS 14% PAGE (A). Eluted Pj10 (1), 20,000 mol. wt (2), 30,000 mol. wt (3) and BSA (4), as control, were then immunoadsorbed either with AC/15.1 MAb-Sepharose (B) or HE2/1 MAb-Sepharose, previously incubated with *P. judaica* sensitive serum pool (C).

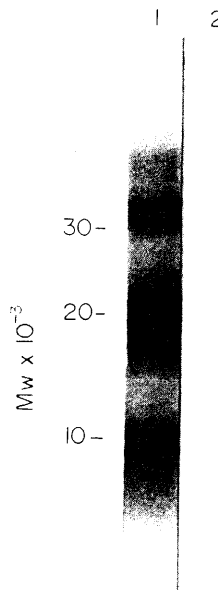


Fig. 6. Immunoadsorption on anti-human IgE-Sepharose. Fifty microliters of HE2/1 MAb-Sepharose was incubated with 1 ml of a *P. judaica* sensitive serum pool for 16 hr. After extensive washing, 5×10^5 cpm of ^{125}I -major allergen (previously precleared on HE2/1 MAb-Sepharose) was added. One hr later, beads were washed, boiled in Laemmli's sample buffer and centrifuged. Supernatants were subjected to SDS-14% PAGE with further autoradiography (lane 1). A control experiment was run by using serum from non-allergic individuals (lane 2). Molecular wt was determined as in Fig. 4.

an allergenic epitope. The monoclonal antibody was able to inhibit no more than 20% of the total IgE-binding ability (unpublished results), as expected from the anti-allergen MAb screening procedure (Corbi *et al.*, 1985a). Although not a conclusive result, it suggests different allergenic determinants are located on the *P. judaica* pollen major allergen. This result resembles those obtained by Elsayed and Apold (Elsayed and Apold, 1983) for cod allergen M.

On the contrary, if the four polypeptides were not bound by non-covalent interactions, it could be concluded that AC/15.1 MAb is directed to an epitope located on a group of proteins with IgE-binding ability. However, cross-immuno-electrophoretic precipitation appears to suggest this is not the case (Corbi and Carreira, 1984). Whichever explanation is correct, AC/15.1 MAb provides a very useful tool for the structural analysis of the allergenic components from *P. judaica* pollen. It has allowed large-scale purification of the native allergen for standardization of *P. judaica* pollen extract (Corbi *et al.*, 1985b) and for biochemical knowledge of the IgE-binding epitopes. At present, we are investigating the presence of common proteolytic peptides in the different structures to explain their antigenic cross-reactivity and the relationship among them under native (non-denaturing) conditions.

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