

The Allergen of Plane-Tree Pollen

Characterization of a Major Allergen

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Isolated allergens are useful tools to study the mechanisms of immediate hypersensitivity and to enlarge our knowledge on humoral and cellular modifications during hypersensitization treatments (5, 6). Isolation of allergens from several origins has been attempted. Six allergens have been described from short ragweed pollen, and the amino-acid sequences of ragweed Ra5 and cod fish allergen M are now available (1,9).

Plane-tree (*Platanus acerifolia*) pollen may induce spring pollinosis in the south of France. We have previously isolated a major allergen from the whole allergenic extract (3). But some other minor allergens could also be found in this same extract. In this paper we studied the release of whole proteins by isoelectrofocusing and of allergen P by radioimmunoassay in the buffer, at different times of extraction. The allergenic activity was tested by RAST.

MATERIAL AND METHOD

Allergen P isolation

Dry plane-tree pollen was purchased from Greer (USA) and defatted for 8 h in a Soxhlet apparatus. Whole allergenic extract and allergen P were obtained as previously described (3).

Extraction at different times

Defatted pollen 25 g was extracted at 4°C in 500 ml phosphate buffer 0.005 M pH 7.2, for 10, 15 and 30 min and 1, 2, 8, 16 and 24 h. The extracts were centrifuged at 8000 g for 15 min at 4°C, dialyzed and lyophilized. The patterns of extraction were determined by isoelectrofocusing using LKB ampholine pag-plate pH 3.5–9.5.

Testing of allergenic activity

Allergenic activity of each extract was tested by direct RAST (10) and pooled sera from sensitive patients were used.

Allergen P radioimmunoassay

Allergen P was measured by double antibody radioimmunoassay using: ¹²⁵I labelled isolated allergen P as described by Yunginger & Gleich (11) for ragweed antigen E, allergen P, specific rabbit antisera and goat anti-rabbit serum.

RESULTS

Isoelectrofocusing

Pollen extracts were obtained after 10, 15 and 30 min and 1, 2, 8, 16 and 24 h. They were dialyzed, lyophilized and an isoelectrofocusing was performed in each case. The patterns of isoelectrofocusing showed that when the time of extraction increased, the numbers of bands also increased, and the staining of the gel became deeper. No difference was noted after 16 and 24 h of extraction.

Allergenic activity

An allergenic activity tested by direct RAST was detected 10 min after the beginning of the extraction, suggesting a fast release of allergenic molecules. The allergenic activity increased until the 8th hour of extraction, and after a plateau was reached (Table 1). Bearing the patterns of isoelectrofocusing in mind, the more basic proteins were released very quickly in the buffer.

Allergen P release

The release of allergen P occurred after 30 min of extraction. The allergen P level reached a maximum after 2 h of extraction, suggesting a slow release of the major allergen. A slight degradation was observed after 16 h of extraction (Table 1).

DISCUSSION

We have previously characterized by isoelectrofocusing and RAST inhibition, a whole allergenic extract of plane-tree pollen, obtained after 24 h extraction at 4°C (2). More than 30 bands were observed. After slicing of the gel and elution of the proteins in saline, the RAST pattern showed three major allergenic activities in 3.5–5.2, 5.8–7.2, and 7.5–8.3 pH ranges.

We first isolated from the whole allergenic extract, a major allergen called allergen P. It is a glycoprotein with a molecular weight of 22 000 daltons and an isoelectric point of 4.2 (3). The allergenic activity was destroyed by heat and proteolytic enzymes, but denaturation by 8 M urea maintained the capacity of this allergen to react with native specific IgE (4).

In this report we have studied the effect of extraction at different times on the release of the whole proteins by isoelectrofocusing, on the allergenic activity and on the release of allergen P. The fast release of some basic

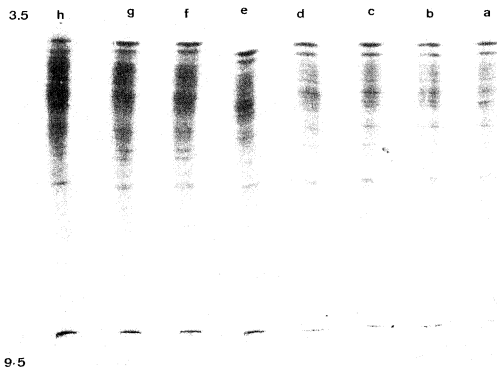


Fig. 1. Isoelectric pattern of extractibility of plane-tree pollen allergens as a function of time: a, 10; b, 15; c, 30 min; d, 1; e, 2; f, 8; g, 16; h, 24 h.

proteins in the medium is related to the appearance of an allergenic activity. This allergenic activity increased during the first 8 h of extraction. Allergen P was released later than these basic proteins, the maximum level being reached after 2 h. Perhaps the very fast release corresponds to other minor allergens which were isolated from plane-tree pollen (2) and with isoelectric

points different from that of allergen P. From short ragweed pollen, Marsh et al. (8) have described a basic protein released very quickly and now characterized as antigen Ra6. Similarly Lowenstein & Mygind (7) reported that some allergens from timothy pollen were rapidly eluted into the buffer.

The role of these rapidly extracted allergens is

Table 1
Allergenic activity assayed by RAST, and plane-tree allergen P release as a function of extraction time

Time of extraction	Allergenic activity ^a RAST (c.p.m. $\times 10^{-3}$)	Allergen P release ^b ($\mu\text{g/ml}$)
10 min	11.5 ± 2.1	—
15 min	14.3 ± 3.5	—
30 min	8.1 ± 0.9	0.3 ± 0.04
1 hr	10.2 ± 1.9	3.9 ± 0.3
2 hr	16.9 ± 3.2	6.2 ± 0.9
8 hr	24.3 ± 3.8	5.8 ± 0.9
16 hr	24.5 ± 4.1	5.5 ± 0.7
24 hr	22.8 ± 3.5	4.7 ± 0.5

^aMean $\pm$ s.d. of 10 experiments.

^bMean $\pm$ s.d. of five experiments.

unclear, but they emphasize the complexity of pollen extracts. The rapidity of extraction of such components would make them important in inducing clinical manifestations in pollen-sensitive patients by reacting with cell bound specific IgE, and allowing the release of mediators.

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Cross Reactions among Pollen Antigens

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The immunochemical relations between antigens originating from different allergenic sources are parameters of crucial importance for the biological characterization of allergenic extracts. Partial immunochemical identity between molecules which share common molecular parts, or complete immunochemical identity between molecules, is easily investigated directly by means of CLIE (7). The type of information obtained is similar to that obtained with the Ouchterlony technique. Four levels of information may be obtained: (1) any immunological identity at all between complex mixtures; (2) immunological identity between one molecule in one mixture and another molecule in another mixture; (3) immunological identity between parts of one molecule and parts of another molecule — the common molecular part comprising several immunological determinants, and (4) im-

munochemical identity between single determinants within the same or from two different molecules.

The information obtained through this technique is related to the probability of patients' allergic reactions to related allergen sources. A simpler immunochemical comparison between related allergenic extracts should include only the allergens but this is not a realistic possibility (1) because of the patients' unique IgE response to complex antigenic mixtures, and (2) because isolated allergens are not generally available. The limitation of this method is the degree of availability of antibodies against all potential allergens. Partial identity may also be determined by means of RAST inhibition, the limitation of which in a similar way is the availability of antibodies against all the potential allergens in the human serum pool used. Preferably the two methods should be used to complement each other. Absence of a

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