

Studies of the allergens of *Olea europea* pollen

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Summary

Allergens of *Olea europea* have been prepared by isoelectrofocusing and tested by RAST, RAST inhibition and skin tests. The major allergen was found to be an acidic protein of pI 6, consisting of two polypeptide chains of molecular weight 15000 and 17000 daltons, possibly non-covalently associated into a dimer. Most other allergens were acidic proteins, but basic proteins were also observed with allergenic activity. The RAST inhibition studies show a considerable degree of allergic cross-reactivity between the isoelectrofocusing fractions.

Introduction

Olea europea (European olive tree) pollen is an important cause of allergic disease in Mediterranean countries. It is estimated that in the south of Spain between 30 and 70% of people with hay fever are allergic to this pollen. Few studies have so far been made on this pollen compared with other inhalant allergens prevalent in Europe, e.g. Timothy (Cramer-Kleinert & Schneider, 1975) and Cocksfoot pollens (Topping, Brostoff & Brighton, 1981).

Purification and characterization of allergenic components from pollen are important for standardization of extracts, and for immunological, epidemiological and genetic studies of the allergic patient.

We describe the characterization and purification of allergens from olive-tree pollen, the major component being an acidic protein of pI 6 consisting of two polypeptide chains of molecular weight 15000 and 17000 daltons respectively.

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Material and methods

Patients

Twenty atopic subjects with a history of hay fever and positive skin tests to a commercial olive pollen extract (Dome Laboratories) volunteered for our studies.

Skin test

Prick tests were made with the crude olive pollen extract at a concentration of 20% (w/v). The various fractions suspended in 50% glycerol saline were tested at a concentration of 50 $\mu\text{g ml}^{-1}$. This solvent was also used as a negative control in skin testing.

Sera

The donor subjects gave sufficient blood for 10 ml of serum to be stored frozen in aliquots. These sera were tested by RAST and those containing most specific antibody were selected for RAST inhibition studies.

Pollen preparation

116.5 gm of pollen grains were defatted in diethyl ether for 30 min at room temperature, filtered, air-dried and extracted with Coca's fluid for 60 min at room temperature. The extract was filtered and centrifuged to clarify (2500 g, 30 min at 4°C). The pellet was discarded and the supernatant concentrated using polyethyleneglycol (Calbiochem, Aquacide, II-A) and dialysed at 4°C in Visking 18/30 tubing against four changes of 0.01 M ammonium bicarbonate and lyophilized. The protein content of the dialysate was measured by absorbance at 280 nm and also by weight of the freeze-dried preparation. The dialysing fluid was similarly examined to determine whether allergenic or antigenic substances had passed through the membrane.

Isoelectrofocusing

Preparative isoelectrofocusing was performed in a Vesterberg-Svensson column (LKB, Broma, Sweden) of 110 ml capacity. The column was stabilized with a sucrose density gradient from 50 to 5% w/v, with the anode at the top of the column, over a pH range of 11 to 2.5. The column was run over a period of 36 h at a temperature of 4°C, at an initial voltage of 350 volts and a final of 1700 volts. The power was limited to 5 watts. At the end of the run 3-ml fractions were collected, their pH and optical density measured at 280 nm.

Radioallergosorbent tests (RAST)

Cellulose paper discs (Whatman 54) 5 mm diameter were activated with cyanogen bromide according to the method of Wide, Bennich and Johanson (1967). Pollen extract was coupled to the disc at a concentration ranging from 1 μg to 10 mg ml^{-1} to determine the optimum. Optimal activity was obtained at 1 mg ml^{-1} . The purified fractions were coupled at 100 $\mu\text{g ml}^{-1}$. Coupled discs were incubated with 100 μl of serum at a dilution of 1 in 15 for 24 h. After washing, the discs were incubated with 50 μl 125 I anti IgE (Pharmacia GB Ltd) for 24 h. The discs were again washed and counted in an LKB γ counter.

Radioallergosorbent test inhibition

The RAST inhibition procedure was a modification of the method of Yman, Ponterius and Brandt (1975). Seven fivefold dilutions of pollen extract (1 to 1/15000) were used, starting at the maximum concentration available, 800 $\mu\text{g ml}^{-1}$. A standard serum concentration was incubated with the pollen solution for 3 h at room temperature and the RAST procedure carried out. A serum from an atopic patient with high total IgE (5000 i.u. ml), but no IgE antibodies to olive pollen was used as a negative control.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

This was performed according to the method of Hudson & Hay (1980). A continuous gradient of 7.5 to 15% acrylamide was used in a vertical slab apparatus. The sample was treated either with mercaptoethanol or iodoacetamide to destroy or protect, respectively, interchain disulphide bonds. Protein markers of known molecular weight (human serum albumin 69000, IgG heavy chain 52000, chymotrypsin 25000, cytochrome C 12500) were used to construct a standard curve to determine the molecular weight of the unknown samples.

Results

Pollen extraction

The 116.5 gm of pollen grains yielded 2.24 gm, i.e. about 2% of the total amount, as crude aqueous freeze-dried extract. The protein estimation by u.v. absorption agreed well with dry-weight determination. The dialysing fluid contained u.v. absorbing material but was devoid of allergen when freeze-dried and reconstituted for skin testing and RAST.

Isoelectrofocusing fractions: skin tests

The profile obtained by isoelectric focusing of crude pollen extract is shown in Fig. 1. This profile was reproducible under the same conditions on several occasions.

Aliquots were grouped into twenty fractions and used for skin testing at a concentration of 50 mg ml^{-1} in the volunteer panel. The allergenic activity of the fractions as a percentage of subjects giving a positive skin test—i.e. a wheal greater

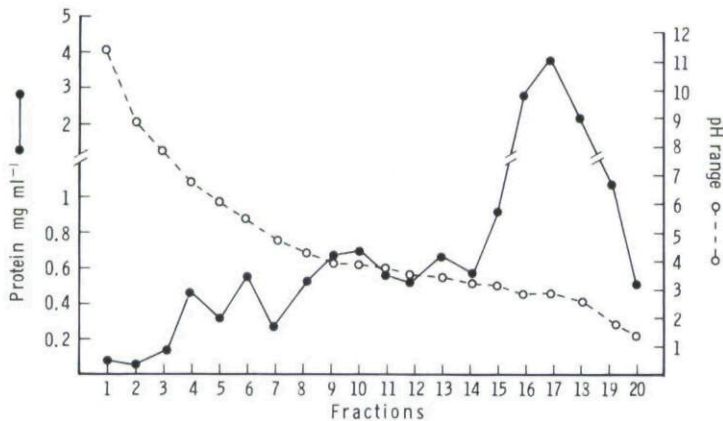


Fig. 1. The isoelectric focusing profile of an olive-pollen extract shows a major protein peak eluted from pH 3.5 to 1.5, but this was not the major allergen.

than 5 mm diameter—is shown in Fig. 2. Fraction 5, of mean isoelectric point 6, is the most frequently allergenic in this group of patients, 95% of whom gave a positive skin test to this allergen. All fractions showed some allergenic activity although not in all the patients studied. Fractions 1, 5 and 8 were chosen for further studies as being representative of basic and acidic proteins of olive pollen

Radioallergosorbent tests (RAST) and RAST inhibition

Whole pollen extract was coupled to the discs and RAST inhibition was performed with the three fractions: 1 (pI 8.5), 5 (pI 6) and 8 (pI 4) at a maximal concentration of 800 μ g. All three inhibited the RAST with similar slopes (Fig. 3). When fractions 1 and 5 were coupled to the discs and inhibition performed, the fractions were found to be equipotent. It can be seen that the slopes of the curves are similar (Fig. 4), indicating a high degree of cross-reactivity between the two fractions.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Figure 5 shows the result of SDS-PAGE of the main allergenic fraction of olive pollen (fraction 5, pI 6). Columns 1 and 3 show the molecular weight markers, columns 2 and 4 consist of fraction 5 treated with mercaptoethanol and iodoacetamide respectively.

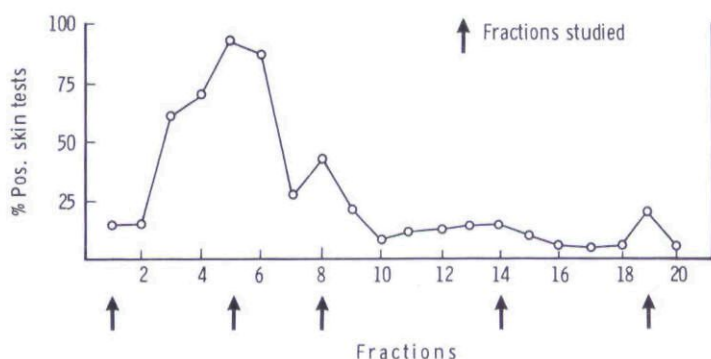


Fig. 2. The major skin-reacting allergenic fractions from isoelectric focusing of olive pollen (Nos 3–6) had pI values from 5.5 to 7.5, the most active being fraction 5, pI 6.

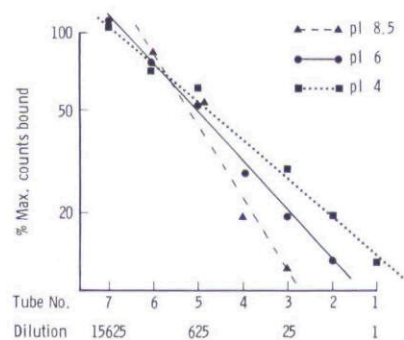


Fig. 3. With whole pollen on the disc, three fractions were tested in RAST inhibition and all showed marked cross-inhibition patterns.

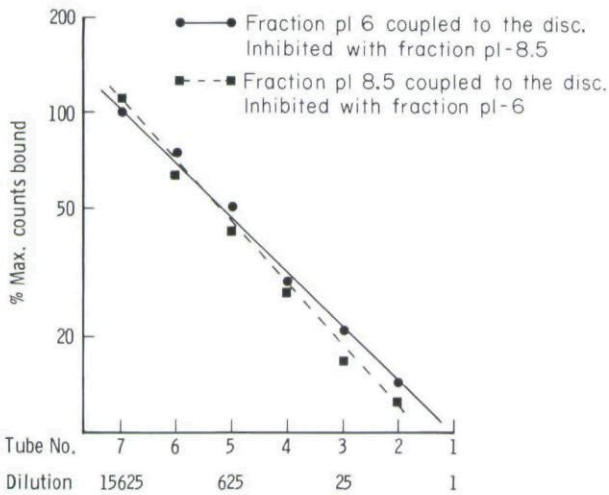


Fig. 4. Fractions pI 6 and pI 8.5 were tested for cross-reactivity in RAST inhibition using each fraction on the solid phase. The inhibition curves were superimposed suggesting similar antigenic determinants in both fractions.

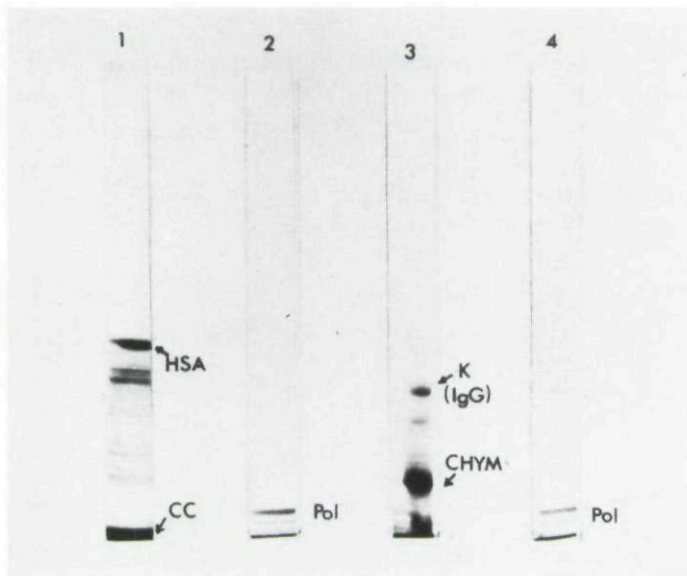


Fig. 5. The SDS-PAGE of the major allergenic fraction from isoelectrofocusing (fraction 5) shows two polypeptides of molecular weight 15000 and 17000 daltons respectively.

The gel revealed two polypeptide chains of molecular weight 15K and 17K. These chains are non-covalently associated with each other but may be associated in the native form but dissociated in the presence of SDS.

Discussion

Several olive-pollen fractions have been defined in terms of skin-test reactivity, isoelectric focusing characteristics, RAST and RAST-inhibition activity. Although all

20 fractions of olive pollen showed some allergenic activity, the main allergen consisted of an acidic protein, fraction 5, pI 6. Other authors have also shown that most *Oleacea* family allergens are in the acidic fractions (Hewitt *et al.*, 1982). Similar results were observed by Topping, Brostoff and Brighton (1981) with cocksfoot pollen, who also found that the two main allergens were acidic with pI values of 4 and 6. Near the pI of the main allergens there are also proteins with allergenic activity: these may be isoallergens of the type described by Roeber, Klapper and Marsh (1982) in ragweed. We and others (Hewitt *et al.*, 1982) have found that there is a considerable degree of cross-reactivity between the allergens of olive pollen including lines of identity on precipitation in gel. Using skin passive sensitization and cross neutralization, Underdown & Goodfriend (1969) have shown similar cross-reactivity with ragweed pollen allergens, in particular between Ra3 and AgE. Griffiths & Brunet (1970) have also found a high degree of cross-antigenicity between AgE and a basic protein (BPA-R) of ragweed. Cross allergenicity has also been noted between the fractions of timothy pollen extract (Cramer-Kleinert & Schneider, 1975). Our results with precipitation show that basic and acidic proteins also cross-react.

The SDS results indicate that our fraction 5, pI 6, has two polypeptide chains and could represent a pure protein of two subunits, having a total molecular weight of 32000. These results contrast with those of Vela *et al.* (1982) who showed by gel chromatography an allergenic fraction of molecular weight 65000. This might represent an aggregate of acidic and basic proteins.

Further studies are in progress to identify all possible allergenic fractions of olive pollen and to confirm by other approaches the molecular weight of the major allergens.

Acknowledgments

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