NATURE OF THE ALLERGENIC ACTIVITY OF POLLEN FROM CERTAIN SPECIES OF PLANTS

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Fractions with allergenic activity, with molecular weight of 37,000, 19,000, 35,000, and 14,000, respectively, were isolated by chromatography from ragweed, wormwood, goosefoot, and sunflower pollen on Sephadex. All the allergens had similar properties. Affinity for Sephadex resins was stronger for Sephadex G-75 than for G-100, the adsorbed components were inactive, and allergenic and antigenic activity was found only in fractions eluted in the bed volume of the column. The affinity of the allergens for Sephadex was independent of the type of eluate but was reduced if the ionic strength of the buffer was increased.

KEY WORDS: pollen allergens; plant pollen; chromatography of pollen.

Knowledge of the clinical nature and properties of the allergens is essential for the study of the mechanisms of development of atopic diseases. However, until recently this problem has received little study [1].

Pollen allergens are of great interest, particularly because most information on the mechanism of development of allergic diseases has been obtained by the use of diseases caused by these allergens as the model. Antigens from pollen of the principal plant allergen, namely ragweed, have been studied in most detail. Antigens of protein–polypeptide nature – E and K [6-8], Ra3 [3, 9], BPA-R [5], and Ra5 [4] – have been isolated from the pollen of this plant. Other pollen allergens have received little study.

In the investigation described below antigenic components of ragweed, wormwood, goosefoot, and sunflower – allergens playing the principal role in the etiology of pollinoses in many of the southern regions of the USSR, were studied.

EXPERIMENTAL METHODS

The pollen was fractionated by chromatography on columns (75 × 2.5 cm) packed with Sephadex G-75 or G-100. The allergens were eluted with 0.15M NaCl solution and 0.1M Tris-HCl buffer, pH 8.05. Fractions 5-6 ml in volume were collected at the rate of 15 ml/h by means of an automatic fraction collector. Absorption of the fractions in the ultraviolet regions was studied by passing the elutate automatically through an LKB Uvicord II absorptiometer at 254 nm and with the SF-4A spectrophotometer at 280 nm.

The allergenic activity of the fractions was determined by scarification tests on patients with increased sensitivity to the pollen of the different plants studied.

Activity of ragweed pollen fractions also was studied by Ouchterlony's gel diffusion method in agar, using rabbit antiragweed serum.

The molecular weight of the allergenically active component of ragweed, sunflower, wormwood, and goosefoot, was measured on a column with Sephadex G-100, calibrated by proteins of known molecular weight (pepsin, trypsin, ovalbumin, and cytochrome c). The homogeneity of the allergenic fractions was studied by electrophoresis in 7% polyacrylamide gel by the method described in [2].

EXPERIMENTAL RESULTS

Chromatography of ragweed, sunflower, wormwood, and goosefoot pollen on Sephadex G-75 and Sephadex G-100 yielded 3-6 components (Fig. 1). The elution volumes of the last fraction of all the allergens studied

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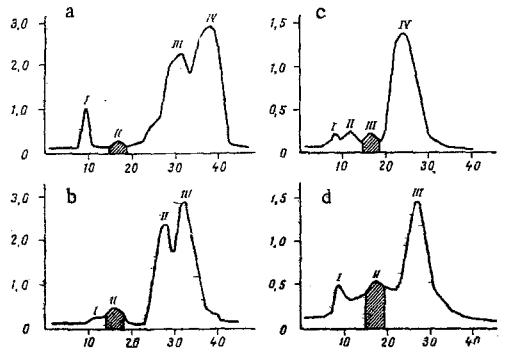

Fig. 1. Chromatograms of extracts of ragweed (a), wormwood (b), goosefoot (c), and sunflower (d) pollen on a Sephadex G-100 column measuring 75 × 2.5 cm. Abscissa, fraction Nos.; ordinate, extinction at 280 nm.

were found to exceed the total volume of the chromatographic columns. This indicates that the pollen allergens had affinity for Sephadex. The reason for this was evidently that the pollen contained large quantities of aromatic and heterocyclic compounds, which are known [2] to possess affinity for Sephadex resins. The affinity of the allergens for Sephadex G-75 was stronger under these conditions than for Sephadex G-100. The profile of the chromatogram was unchanged if the 0.15M NaCl solution was replaced by 0.1M Tris-HCl buffer. However, if the polarity of the buffer was increased to 0.5M, the degree of adsorption of the allergens was reduced, with a consequent reduction in the volumes. The last fractions of all allergens contained pigment.

Characteristically only those components of the pollen which were eluted within the bed volume possessed allergenic activity. The adsorbed components possessed no activity. All fractions with allergenic activity were electrophoretically nonhomogeneous and contained as many as two to four components.

Fraction II of ragweed pollen, fraction III of goosefoot pollen, fraction II of wormwood pollen, and fraction II of sunflower pollen possessed the strongest allergenic activity. All the antigenic activity of ragweed pollen, and likewise all its allergenic activity, were shown to be due to fraction II.

The comparison of the elution volumes of the allergenically active action with the elution volumes of proteins of known molecular weight showed that the molecular weight of the pollen allergens were approximately as follows: ragweed 37,000, wormwood 19,000, goosefoot 35,000, and sunflower 14,000.

These results must be taken as somewhat approximate, for the possibility of adsorption of fractions with allergenic activity on the Sephadex cannot be ruled out. However, they confirm the view that plant pollen allergens have in most cases a molecular weight of between 10,000 and 40,000 [3-9].

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