

Note

Purification of allergens by high-performance liquid chromatography

VI. Purification of the major allergen from *Parietaria judaica* pollen

NAZARIO RUBIO*

Instituto Cajal, Velázquez 144, 28006 Madrid (Spain)

and

AURORA BRIEVA

Laboratorios Andromaco S.A., Allergy Division, Torrejón de Ardoz (Spain)

(Received June 16th, 1987)

Size-exclusion high-performance liquid chromatography (HPLC) is an important method for protein separation. It is now one of the more useful techniques for the purification of biologically active macromolecules. We have previously purified several high-incidence allergens from different sources by HPLC¹⁻⁵.

Parietaria judaica is responsible, in addition to olive pollen, for an high percentage of allergies in the mediterranean area⁶. The purification of the major allergens from both species will contribute to the improvement of the treatment of those patients, suffering from these allergies.

We now report the purification of the main allergen of *P. judaica* pollen in a single stage using Protein Pack 125 HPLC columns.

EXPERIMENTAL

Allergen extraction

Pollen from *Parietaria judaica* was obtained from Allergon AB (Angelholm, Sweden). Extractions were performed at 5% (w/v) in phosphate-buffered saline (PBS) containing 50 µg of phenylmethylsulphonyl fluoride (PMSF) for 1 h in an ice-bath.

HPLC

HPLC was performed as described previously¹⁻⁵.

Immunological techniques

Solid-phase radioimmunoassay (SPRIA) and radioallergosorbent test (RAST) inhibition were carried out according to refs. 1 and 2.

Sera

Sera selected from patients who had clinical histories of wall pellitory (*P. judaica*) allergy were provided by Dr. A. Olive, Hospital Sant Pau, Barcelona. All sera were screened with RAST and showed specific immunoglobulin E (IgE) binding (RAST class 4). Sera were stored frozen at -20°C.

NOTES

RESULTS AND DISCUSSION

The HPLC purification of *P. judaica* allergen can be performed in one step in 20 min. PBS, the usual mobile phase used by us, provides a convenient medium for the separation depicted in Fig. 1. The chromatogram looks very heterogeneous. This is partially due to the presence in the crude extract of large amounts of green pigments. The 10% (w/v) extracts are too dense to filter and inject, and 5% extractions are currently used with this pollen.

When three different sera selected for high activity in RAST against *P. judaica* discs were used to check the presence of specific IgE-binding activity, the histograms in the lower part of Fig. 1 were obtained. A study of the fractions seems to indicate a gaussian distribution with a peak of activity in fraction 9 (shaded area in Fig. 1).

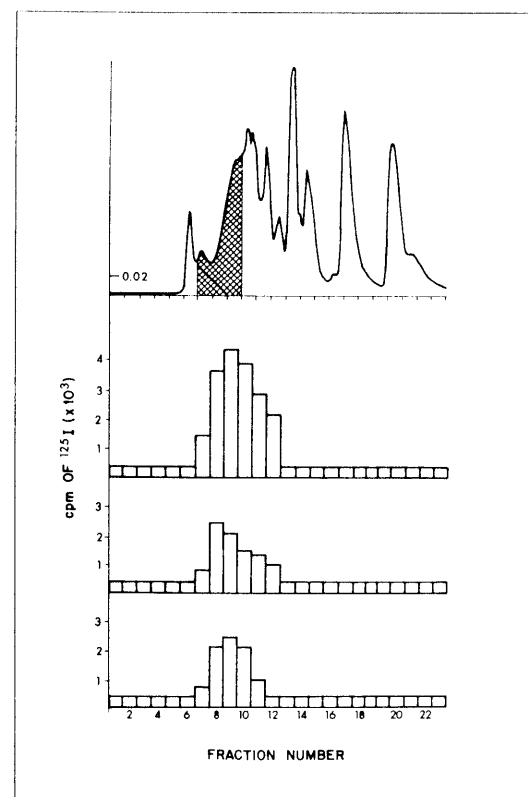


Fig. 1. HPLC of a crude extract of *Parietaria judaica* pollen. Three human sera (RAST class 4) were used in the SPRIA analysis of the different fractions.

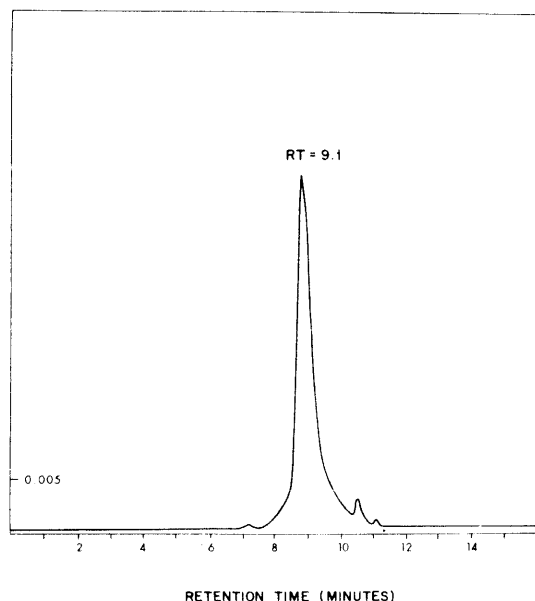


Fig. 2. Rechromatogram of HPLC-purified fraction 9. Conditions as in Fig. 1.

Rechromatography of fraction 9, using the same conditions and higher sensitivity of the UV detector, demonstrates that the allergen in this fraction is present in an almost pure form. Integration by the data module shows that the allergen, with a retention time of 9.1 min, is 98.2% pure (Fig. 2).

Previous reports by other authors have demonstrated that the *P. judaica* allergen contains disulphide bridges under non-reducing conditions, appearing as a dimeric material with a molecular weight of 20 000⁷. Reducing agents, such as β -mercaptoethanol, yield a polypeptide of molecular weight 10 000 in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The mild conditions used by us in the HPLC separation explain the position in the chromatogram (t_R = 9.1 min) which corresponds to a molecular weight of around 20 000 daltons, as a consequence of the aggregation to dimers. SDS-PAGE of this material on boiling for 1 min in 1% β -mercaptoethanol yielded a single band of MW = 10 000 (not shown).

The allergenic activity of the purified allergen was compared with the Pharmedgen[®] extract by RAST inhibition. Both products shows identical potencies, as demonstrated in Fig. 3.

When a quantitative calculation was performed, taking into consideration the amount of protein contained in the HPLC-purified allergen and the biological activity of the Pharmedgen[®] extract, we found that 3.4 ng are needed to produce 1 Biological Unit. This also demonstrates that our HPLC purification procedure completely retains the IgE-binding ability of the allergenic molecule.

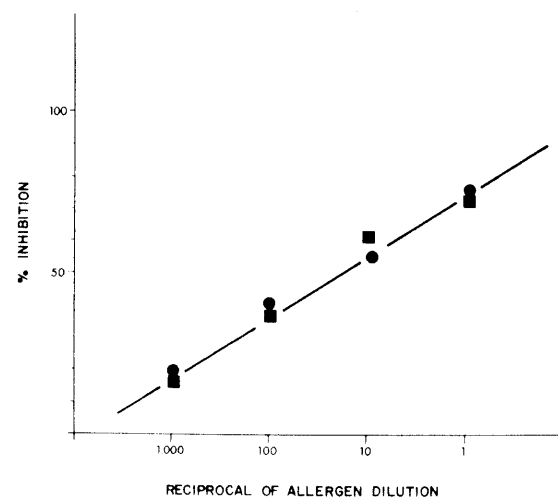


Fig. 3. RAST inhibition assay. The undiluted Pharmedgen[®] extract corresponds to 100 000 Biological Units/ml. ■—■, HPLC-purified allergen; ●—●, Pharmedgen[®] extract.

The speed and recovery are the main advantages of the HPLC purification of allergens. *P. judaica* main allergen has already been purified by biochemical techniques^{6,8} and immunoabsorption using monoclonal antibodies⁹. Here, we stress the fact that the pure major IgE-binding protein from wall pellitory can be obtained in 20 min with all its biological activity fully retained. This method will provide pure material for the therapy of patients from southern and western european countries sensitive to *Parietaria judaica*.

ACKNOWLEDGEMENT

We are indebted to Miss Mercedes Alonso for her technical assistance.

REFERENCES

1. A. Brieva and N. Rubio, *J. Chromatogr.*, 370 (1986) 165.
2. N. Rubio, A. Brieva and M. Rubio, *J. Chromatogr.*, 392 (1987) 447.
3. N. Rubio and A. Brieva, *J. Chromatogr.*, 398 (1987) 366.
4. N. Rubio, A. Brieva and B. Alcazar, *J. Chromatogr.*, 403 (1987) 312.
5. N. Rubio and A. Brieva, *J. Chromatogr.*, 404 (1987) 378.
6. S. Feo, R. Cocchiara and D. Geraci, *Mol. Immunol.*, 21 (1984) 318.
7. A. L. Corbi and J. Carreira, *Int. Arch. Allergy Appl. Immunol.*, 74 (1984) 318.
8. P. Falagiani, E. Cavallone, M. Nali, B. Rindone, S. Tollary and G. Crespi, *J. Chromatogr.*, 328 (1985) 425.
9. A. L. Corvi, V. Ley, F. Sanchez-Madrid and J. Carreira, *Mol. Immunol.*, 22 (1985) 1081.