

Allergens from rye pollen (*Secale cereale*)

II. Characterization and partial purification

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Rye pollen was incubated for 30 min and proteins extracted at this time were collected as extract A (EA). The same pollen grains were resuspended in buffer and incubated for 18.5 h. Proteins extracted in this period were designated extract B (EB). Both extracts were subfractionated by DEAE ion-exchange chromatography and allergen presence in peaks detected by the dot-immunobinding technique. The results reveal that unretained proteins (peaks 1 and 2) and proteins eluted at 0.2 M NaCl from extract B contain the highest proportion of allergens. SDS–PAGE of chromatographic peaks showed that peak 2 from extract B contains a highly purified 28 kDa band. On the skin of allergic patients this band gave a stronger positive prick test than for the crude extract.

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Characterization and purification of allergens in extracts obtained from allergenic pollens are an essential prerequisite for understanding the basis for allergic sensitization and an important step towards successful hyposensitization therapy (11).

Rye pollen is an allergen source causing Type I hypersensitivity reactions, and the characterization and purification of these allergens have been studied (1–10, 12, 13).

In a previous paper, we reported that the maximum allergen proportion for rye pollen was collected between 30 min and 19 h of pollen incubation (10). In this paper we have utilized this knowledge to produce two extracts of rye pollen which were used to study biochemical features of its principal allergens and to partially isolate one of them by a simple procedure.

Material and methods

Extract preparation

Three grams of rye pollen were resuspended in 60 ml of 0.01 M Na₂HPO₄–KH₂PO₄ pH 7.4, 0.15 NaCl (PBS) and gently incubated for 30 min at 4–8°C. The suspension was centrifuged (8700 × g) for 10 min in a SS 34 Sorvall rotor. The supernatant was filtered successively through 0.45 µm and 0.22 µm Milipore filters and finally frozen at –70°C. This extract was designated extract A (EA). The pellet was resuspended in 60 ml of PBS and the incubation was continued a further 18.5 h with centrifugation and supernatant filtering as described above at the end of the process. This extract was designated extract B (EB).

Ion-exchange chromatography on DEAE-32 cellulose

A and B extracts were equilibrated against 150 mM Tris HCl pH 8. 60 mg EA and 100 mg EB were subfractionated under the same conditions in a Whatman DEAE-32 cellulose column of 1.8 × 19 cm equilibrated with the same Tris buffer. Protein elution was performed in stepwise ionic strength gradient produced by sequential increase of NaCl molarity in the elution buffer (10 mM, 50 mM, 100 mM, 200 mM, and 500 mM, successively).

Protein evaluation, SDS–PAGE and dot-immunobinding assay were performed as described in previous paper (10).

Skin test

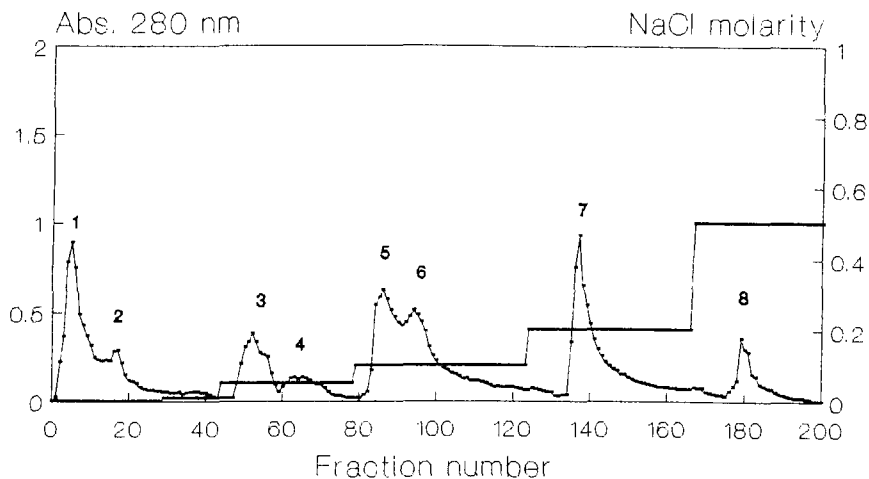
Peak 2B was dialysed against PBS. A drop from whole extract and from peak 2B (with the same protein concentration in both, 0.1 mg/ml) were used in the skin prick test on 15 patients sensitized to rye pollen proteins. After 15 min wheal diameters were measured and compared.

Results and discussion

Extracts A and B were subfractionated under the same ion-exchange chromatography conditions and the eluted peaks compared. Fig. 1 shows the respective elution profiles. Both processes produced the same number of peaks, but the proportions of peaks 1, 2, 7 and 8 were significantly higher in EB.

Results from dot-immunobinding (Fig. 2) indicate

EXTRACT A (First 30 minutes)



EXTRACT B (From 30 min to 19 h)

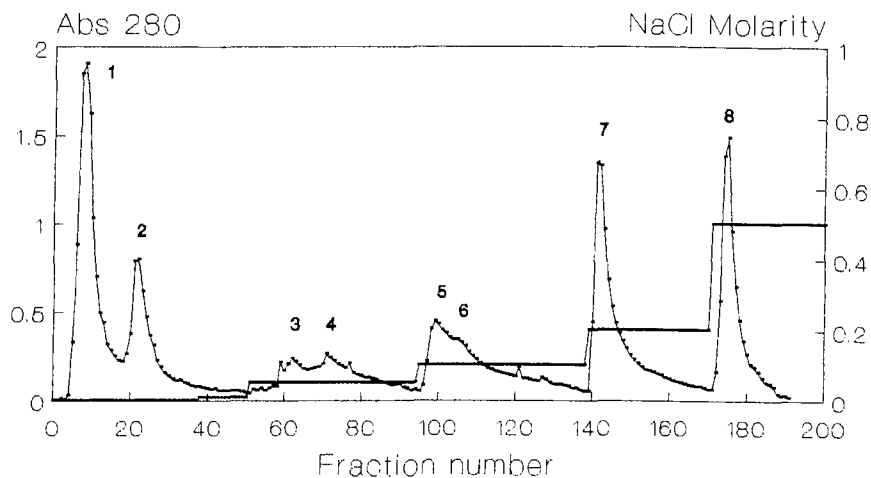


Fig. 1. Ion-exchange chromatography of extracts A and B on a DEAE-32 column eluted with a step-wise ionic strength gradient. Numbers identify the peaks posteriorly studied.

that most of the sera from allergic patients bound IgE in a high proportion to basic proteins (peaks 1 and 2) and particularly in that from EB. These results concur with those observed by Löwenstein et al. (8) and Renck et al. (12), using CRIE techniques. Westphal et al. (13), describe some acidic proteins as major allergens by immunoreaction of allergic serum with *Secale cereale* proteins previously separated by isoelectrofocusing technique and transferred

to nitrocellulose paper. Nearly all the proteins in their study are focused in the acidic range and no basic ones were seen, even though some of their patients showed some reactivity to them. Our own experience shows that basic proteins are barely focused. This could explain the discrepancy in the results.

Peak 7 was highly reactive in EB while in EA it showed almost no reactivity. One patient (No. 11),

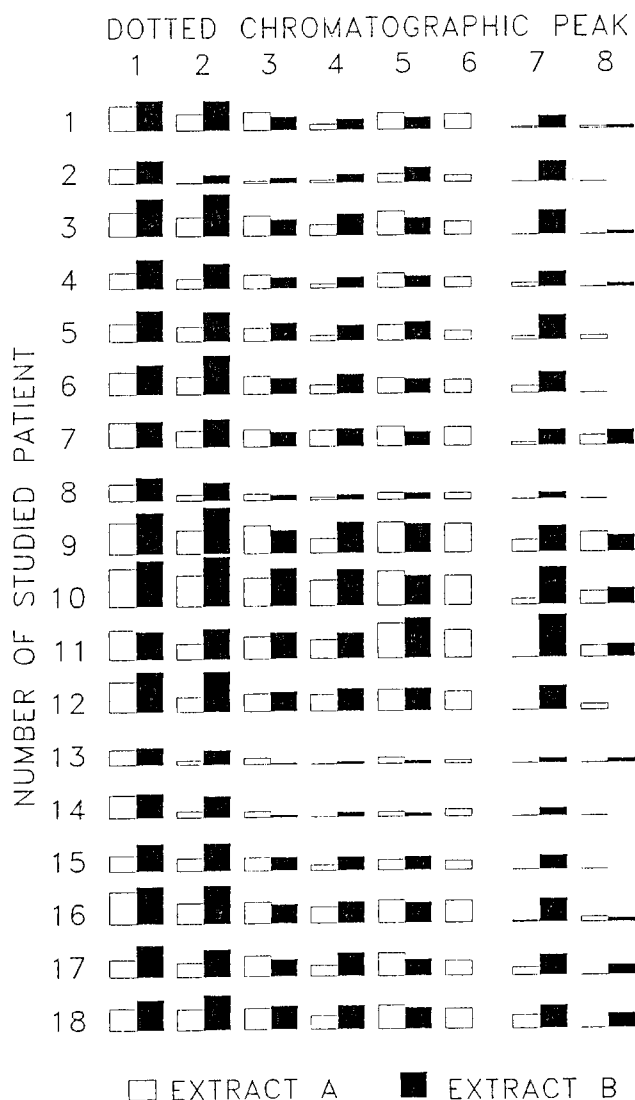


Fig. 2. Graphic bar representation of values obtained in the dot-immunobinding techniques for detecting peaks from DEAE-chromatographies having a major proportion of allergens.

showed proportionally less allergens among the basic proteins, and increasing allergenicity with the acidity of the studied fraction, reaching a maximum value in EB peak 7. A similar result is described by Renck et al. (12).

All the other studied peaks bound IgE with values which were always at least two or three times below those for EB peaks 1 and 2. Peak 8 was practically non-reactive in both extracts. Peak 6 from extract B was not considered as an independent peak in this study.

In a previous paper (10) we reported that major allergens from rye pollen had molecular weights of 28 kDa, 33 kDa, 48 kDa and 67 kDa. In this work we looked for them between the chromatographic peaks by SDS-PAGE analysis of rye pollen proteins (Fig. 3). We found that the band of 67 kDa was

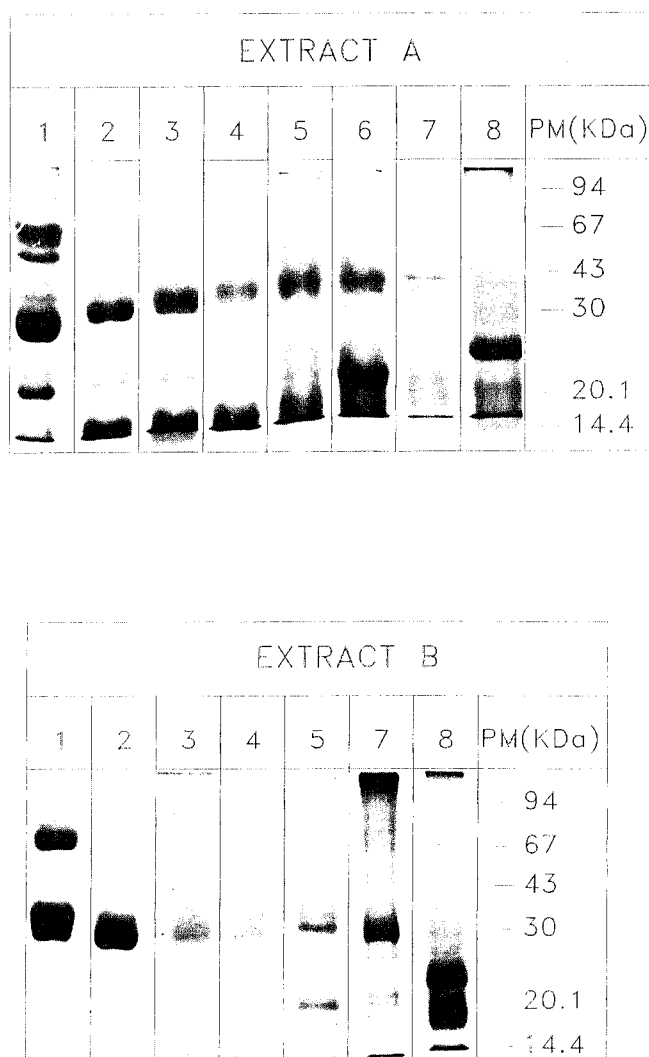


Fig. 3. Protein-stained profiles of peaks from EA and EB chromatographies after SDS-PAGE. Numbers identify the studied peak.

collected only in the first peak from both extracts (basic proteins). The band of 48 kDa was not clearly detected between peaks. Bands of 28 kDa and 33 kDa were present in all the collected peaks, showing differences in proportion and mobility which justify their differences in reactivity. A highly purified band was seen in peak 2 from extract B, and according to the outlined dot-immunobinding results, peak 2 from extract B could contain a highly purified allergen.

To verify this a prick test was carried out in 15 allergic patients. All patients had a positive skin test with a similar or superior intensity than the total extract (Fig. 4). Results of this work reflect that after 30 min an important fraction of released protein comprises allergens which are principally eluted in peaks 1, 2 and 7.

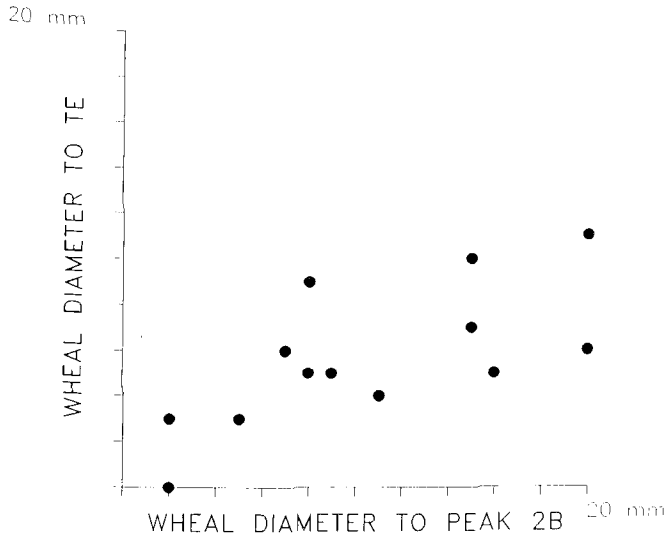


Fig. 4. Relation between wheal diameter (mm) developed for TE and peak 2B proteins, in SPT on each studied patient.

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References

1. JELKE H, WESTPHAL W, BECKER WM, SCHAAK M. Immunoprint technic: a new method for planned production of allergen-specific monoclonal antibodies. *Infection* 1989; 17: 55-7.
2. JORDE W, LINSKENS HF. Versuche zur Isolierung des Roggen-pollenallergens. *Z Immun Allerg Forsch Suppl.* 1974; 214-20.
3. KALVERAM KJ, FORCK G. Untersuchungen an Pollinosis Patienten mittels Hauttest, RAST und RAST-inhibitions-test zur Frage der Antigenverwandtschaft bei Gräser-und Roggen-pollen. *Arch Dermatol Res* 1977; 260: 17-27.
4. KALVERAM KJ, FORCK G. Cross-reactivity between grass and corn pollen antigen. *Int Arch Allergy Appl Immunol* 1978; 57: 549-53.
5. LINSKENS HF, JORDE W, VAN DER WERKEN P. Time dependent release of allergens from rye pollen (*Secale cereale*). Application of a quick extraction to the biomedical analysis of allergenic activity. *Allergol Immunopathol* 1977; 5: 332.
6. LINSKENS HF, VAN DER WERKEN P, JORDE W. The information of allergens during the development of rye pollen (*Secale cereale*). *Allergol Immunopathol* 1980; 8: 35-42.
7. LOEWE G, SCHEIBE F, LUDWING C, DIEZEL W. Zur säulen-chromatographischen Fraktionierung von Roggenpollen-allergenen (*Secale cereale*). *Arch Otorhinolaryngol* 1978; 220: 227-86.
8. LÖWENSTEIN H, WIHL JA, BACHE BILLESBÖLLE K, BÖWADT H. Rationale for specific immunotherapy of grass pollen allergy with extracts of rye pollen. *Allergy* 1984; 39: 421-32.
9. MATTHIESEN F, KLYSNER S, LÖWENSTEIN H. Characteristics of grass pollen allergens. In: *Epitopes of atopic allergens. Proceedings of Workshop held under the aegis of the XIVth Congress of the European Academy of Allergy and Clinical Immunology*. Berlin, September, 1989.
10. MONTERO MT, ALONSO E, SAINZ T. Allergens from rye pollen (*Secale cereale*). I. Study of protein release by rye pollen during a 19-hour process. *Allergen identification. Allergy* 1992; 47: 22-25.
11. PRZBILLA B, RING J. Diagnostik und therapie der allergie vom Sofort-typ gegen Bienem-und Westpengift. *Allergologie* 1985; 8: Suppl. 1: 31-6.
12. RENCK B, EINARSSON R. Crossed radioimmuno-electrophoretic analysis of cultivated rye (*Secale cereale*) pollen allergen. *Int Arch Allergy Appl Immunol* 1987; 83: 96-103.
13. WESTPHAL W, BECKER M, SCHLAACK M. Analysis of rye pollen (*Secale cereale*) allergens using patient IgE, immunoprint, Westernblot and monoclonal antibodies. *Int Arch Allergy Appl Immunol* 1988; 86: 69-75.

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