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Characterization of Birch Pollen Allergens by Immunological Methods

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Birch pollen extract was prepared by extraction of 1.0 g non-defatted pollen (*Betula pendula seu verrucosa*) in 10 ml phosphate-buffered saline (pH 7.2) by mild shaking for 24 h and subsequent centrifugation at 22 000 g for 20 min at 4°C (10). The protein content and the allergenic activity of this extract were 7.9 mg/ml and 100%, respectively. Rabbit antiserum against birch pollen was raised and used in quantitative immunoelectrophoretic methods as described by Axelsen et al. (2). The crossed immunoelectrophoretic (CIE) precipitation pattern obtained at pH 8.6 in agarose gel revealed nine different antigens. No cathodically migrating antigens were observed. Crossed radioimmunoelectrophoresis (CRIE) indicated that IgE from patients allergic to birch had affinity for the major allergens (antigens Nos. 2 and 7/8). Fractionation of the allergen-containing extract by Sephadex G-100 gel filtration was followed by fused rocket immunoelectrophoresis (2) or enzyme-immunoassay (EIA; 9, 11) to obtain the immunochemical elution profile (Fig. 1). Antigens eluted in protein peaks A, B and C, with mean molecular weights of 79 000, 29 000 and 17 000 daltons, respectively, were the most effective in binding rabbit antibodies in EIA. Two thermostable (TS) antigens (Nos. 7/8, mw 10 000-20 000) and a thermolabile one (No. 2, mw 29 000) were shown by CIE and fused rocket immunoelectrophoresis. These results are in agreement with those of Belin (4). The concentration of the TS antigens in the pooled protein peaks was determined by rocket immunoelectrophoresis and the allergenic activity was shown both by prick skin testing and by the RAST inhibition method (12). The results are given in Table 1.

Pooled peak C fractions (mw 10 000-20 000; Fig. 1, Table 1) containing the main allergenic activity showed about 5-fold increase in the concentration of the TS antigen and about 2- to 5-fold increase in the specific allergenic activity (per µg of protein) when compared to the whole extract (9). About 30% of the total allergenic activity was recovered in the peak C fractions and less than 0.5% outside these fractions.

The specific allergenic activity of the peak B fractions was 10-300 times lower than that of the peak C fractions by prick skin testing and by the RAST inhibition method, respectively (9). The non-parallelity of the inhibition curves and the antigenic differences in CRIE indicate that peak B contains allergens distinct from those of peak C. In addition, peak B certainly contains some of the allergens of peak C as cross-contaminating antigens.

The fractions of peak D showed about 1000-3000 times lower specific allergenic activity than those of peak C by prick skin testing and by the RAST inhibition, respectively. Parallel inhibition curves indicate either qualitative allergenic similarities between the allergens in these fractions or cross-contamination (1:3000) of peak D fractions with peak C allergens (1, 9).

Our data do not allow a clear distinction of the origin of the allergenically active components of peak D fractions. The amount of peptides was doubled during the long extraction (3-24 h, at 4°C) suggesting that also the active components may be degradation products of the allergens. Birch pollen allergens are sensitive to many proteinases, especially pronase, but relatively resistant to carbohydrases and to some proteinases, e.g.

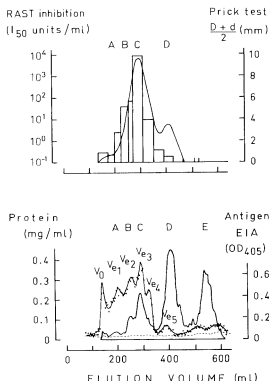


Fig. 1. (Reproduced from Viander et al. (9)). Sephadex G-100 gel filtration of birch pollen extract (5 ml, 10^{-1} w/v). Protein concentrations of the fractions (peaks A, B, C, D and E), are shown by solid line (—). Binding of rabbit antibodies to the different fractions (EIA, absorbancy at 405 nm = OD_{405} ; peaks Ve_0 , Ve_1 , Ve_2 , Ve_3 , Ve_4 and Ve_5) are shown by dots connected by a line (—•—). Background binding of antibodies from normal rabbit serum is indicated by dotted line (----). The upper part of the figure shows results of prick tests (open columns, mean of 10 individuals) and of RAST inhibition (solid line) using pooled fractions corresponding to the various protein peaks. A mixture of sera from 20 allergic subjects was used for the RAST inhibition.

trypsin and pepsin (7). Our results show that even if some proteolysis had occurred during the long extraction, it did not markedly destroy the main allergens of birch pollen, since the allergenic activity in the peak C fractions and the amount of the TS antigen in the extract were increased during the extraction. More than 70% of the thermostable antigens and of the total allergenic activity were released during the first 30 min of extraction. These findings are in agreement with those of Belin (3), who demonstrated the extraction of the major part of the TS antigens and the main allergens

of birch pollen during the first 45 min under similar conditions as described here.

A good correlation was observed between the TS antigen content and the RAST inhibition results. However, the allergenic activity observed by the RAST inhibition method was generally lower than that by prick skin testing, except for the peak C fractions. A possible explanation of this discrepancy may be different binding of the allergens to the paper discs (5). A preferential binding of the main allergens (peak C) would give a misrepresentatively high allergenic activity for the allergens in peak C in relation to the allergens outside these fractions. Thus the coupling of the whole allergenic extract to the solid phase seems to be a critical step, when the RAST inhibition assays are used, for the estimation of the allergenic potency of purified allergens.

RAST inhibition experiments have been used either for the comparison of the total allergenic activity of the extracts (8, 12) or for the demonstration of the cross-reactivity between the purified allergens (1). In this study the inhibition curves were linear between 25% and 75% of inhibition (9). According to Adolphson et al. (1) parallel inhibition curves indicate allergenic identity, and non-parallel curves either cross-reactivity or cross-contamination between the allergens. With these criteria allergens in peak B and C were non-related and those in peak D and C were cross-reactive. The relative allergenic activities obtained for the semipurified allergen preparations (peak C, peak B and peak D) expressed as nanograms of the inhibitor required for a 50% inhibition of the RAST using the whole extract, were 20 ng, 6300 ng and about 55 000 ng, respectively. These are of the same order as observed for highly purified allergens from ragweed pollen using a RAST inhibition method slightly different from the present method; employing only 5 μ l of serum and microcrystalline cellulose as the solid-phase in place of paper discs (1).

Separation of the birch pollen allergens by simple Sephadex G-100 gel filtration yielded only partially purified allergen preparations. Gel isoelectric focusing revealed six protein bands with pIs between 5.6 and 7.2 in the main allergenic fractions. The strongest bands had pIs 5.6 and 5.7, which is of the same order as observed by Ceska et al. (6) for the main allergens of birch pollen (*Betula lenta*). The antigenic properties of the other protein bands remain to be studied. They may represent isoallergic variants of the main allergens or separate allergens eluted in the same fractions.

We have used several immunological methods to characterize the allergens of birch pollen. The combination of the RAST technique with the quantitative immunoelectrophoretic methods proved advantageous in this study. The elution of the separated antigens after Sephadex G-100 gel filtration was easily detected by using rocket immunoelectrophoresis or a more sensitive solid-phase enzymeimmunoassay. Further separation of birch pollen allergens, e.g. by preparative isoelectric

Table 1 (Reproduced from Viander et al. (9))

Allergenic activities and concentrations of the thermostable (TS) antigen of pooled Sephadex G-100 fractions compared to those of the whole birch pollen extract

	Protein µg/ml	Antigen ^a %	Allergenic activity		Specific activity ^d		Recovery ^e	
			%		Prick test	RAST inh.	%	
			Prick test ^b	RAST inh. ^c			Prick test	RAST inh.
Whole extract (10 ⁻³ w/v) (5 ml)	7900	100	100	100	1	1	100	100
Peak A (fr. 37-51)	13	0	0.01	0.0001	0.05	0.001	0.16	0.0025
Peak B (fr. 58-64)	55	0	0.19	0.004	0.45	0.006	0.09	0.028
Peak C (fr. 69-77)	131	10.9	3.3	3.04	4.5	1.84	30.1	27.8
Peak D (fr. 97-104)	330	0	0.01	0.003	0.004	0.0007	0.1	0.027

^a The concentration of thermostable (TS) antigen measured by rocket immunoelectrophoresis (% of whole extract, 10⁻¹ w/v).

^b Mean allergenic activity (%) relative to that of the whole extract (10⁻³ w/v) measured by skin prick testing in 10 allergic individuals.

^c Expressed as $\frac{1_{50} \text{ units/ml of the fractions}}{1_{50} \text{ units/ml of the whole extract}} \times 100$ (%). Mean results of two separate determinations varied less than 5 %.

A mixture of sera from 20 allergic subjects was used.

^d Allergenic activities relative to the amount of protein of the fractions compared to that of the whole extract; the activity of the whole extract was arbitrarily chosen to be 1.

^e Recovery of the total allergenic activity of the various protein peaks as percentages of that of the whole extract.

focusing or by affinity chromatography, should be performed to obtain immunochemically pure allergen preparations.

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