

Investigation of the Allergenic Activity of Isolated Fractions of a Standardized Partly Purified Whole Mite Body (*Dermatophagoides pteronyssinus*) Extract

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Abstract. Twelve fractions of a molecular weight range of 1.35–670.00 kilodaltons (kD) were isolated from a biologically standardized partly purified whole mite body extract (*Dermatophagoides pteronyssinus*) by preparative size exclusion high-performance liquid chromatography. The allergenic activity and the antigen and allergen patterns of the isolated fractions were investigated by RAST, RAST inhibition and crossed (radio)immunoelectrophoresis (CIE/CRIE). By CRIE, each of the fractions showed allergen patterns, mostly of different compositions. Each fraction showed allergenic activity of different degrees by RAST inhibition. The highest allergenic activity could be measured by RAST inhibition with fractions which contained predominantly the major allergens Der pI and PY as detected by CRIE. Also proteins of higher molecular weights (> 158 kD) showed IgE-binding capacities. Nearly all antigens detected by CIE could be identified as allergens using CRIE.

Introduction

Allergen extracts are used in human medicine for the diagnosis and therapy of immunoglobulin-E-mediated allergic diseases. They are prepared as aqueous solutions from materials of biological origin, e.g. animal epithelia, pollens or house dust mites. They contain all water-soluble components of these raw materials. Most of the allergenic components are proteins and glycoproteins with different isoelectric points and molecular weights. By crossed radioimmunoelectrophoresis [1] and immunoblot [2–4], it can be determined which of the proteins of an allergen extract are allergens. By using the sera of almost 15–20 allergic patients they can be classified as major, intermediate and minor allergens [5, 6].

Most of the major allergens of allergen extracts are found in a molecular weight range of approximately 10–60 kilodaltons (kD) [7], but in some cases allergens of higher molecular weights could be identified, mostly of intermediate and minor character. In orchard grass pollen extracts, Ford et al. [8] detected allergens with molecular weights of > 669.00 kD. Allergenic components of the cockroach could be identified in a range of molecular weights of

26.00–120.00 kD [9]. Stewart et al [10] found an allergen of a molecular weight of 244.00 kD in a *Dermatophagoides pteronyssinus* extract (Dpt 4). Recently Baldo et al. [11] identified allergens of whole mite body extracts (*D. pteronyssinus*) in a molecular weight range > 110.00 kD by protein blotting.

In the position paper on immunotherapy [12], it was recommended that allergen extracts should contain all allergens, which are of relevance for the patients. We investigated the antigen and the allergen patterns and the total allergenic activity of several fractions of a mite, *D. pteronyssinus*, extract isolated by size exclusion high-performance liquid chromatography (SE-HPLC). Special consideration was attached to the question whether proteins of high molecular weights display IgE-binding capacities.

Material and Methods

Twelve fractions of a biologically standardized partly purified whole mite body allergen extract (*D. pteronyssinus*) [13, 14] were isolated by preparative SE-HPLC and were stored lyophilized. Each fraction was investigated separately by radioallergosorbent tests (RAST). RAST inhibition and crossed (radio)immunoelectrophoresis (CIE/CRIE).

Preparative SE-HPLC

Preparative SE-HPLC was done as described previously [15]. During each run, 30 mg of protein [16] were injected, and 11 runs were performed. Prior to the investigation, the system was calibrated with a test kit containing a mixture of proteins ranging from 1.35 kD to 670.00 kD (Bio Rad Labs, Munich, FRG).

CIE/CRIE

The antigen/allergen patterns of the isolated fractions were determined by CIE/CRIE [1, 17] using a rabbit anti-*D. pteronyssinus* antiserum (ALK, Copenhagen, Denmark). From each fraction, 10 µg protein were applied onto the gel (60 µl of antiserum in 1 ml agarose). For CRIE, a serum pool ($n = 21$; RAST class 4 using *D. pteronyssinus* allergen disks) of mite-allergic patients (1:24 diluted) was used. The allergen patterns were made visible by autoradiography (3 kBq/ml of ^{125}I -antihuman IgE (Pharmacia, Freiburg, FRG)) on Kodak X-Omat AR films. The time of autoradiography was 3, 7 and 14 days at -20°C . For the identification of the antigen pattern, the CRIE plates were stained with Coomassie brilliant blue R 250 (Merck, Darmstadt, FRG) after autoradiography.

RAST

To investigate whether all isolated fractions yield a positive RAST, the sera of 8 mite-allergic patients (positive skin test with a *D. pteronyssinus* prick test solution; Allergopharma, Reinbek, FRG) with a positive clinical history and a pool serum of nonallergic patients ($n = 43$) were measured by RAST using allergen disks [18] prepared with the isolated fractions of the extract. For comparison, allergen disks prepared with the unfractionated extract were included into the investigation.

RAST Inhibition

For the determination of the allergenic activity by RAST inhibition [19] 5 ml of each fraction were lyophilized and then dissolved in 2.5 ml doubly distilled water. Different dilutions (1:3 steps) of each allergen extract were incubated in test tubes (12 × 55 mm) with a serum pool prepared with sera of mite allergic patients (RAST class 3–4) undergoing biological standardization ($n = 21$; 1:10 diluted). The mixtures were incubated overnight at room temperature with mite allergen disks, which were prepared with the nonfractionated biologically standardized mite (*D. pteronyssinus*) allergen extract. Subsequently, after washing steps, rabbit ^{125}I -antihuman IgE (Fc-specific; Pharmacia) was added as tracer for IgE. After a sec-

ond overnight incubation, the allergen disks were washed carefully and counted in a gamma counter for bound radioactivity. The non-fractionated extract was calculated as reference. For the calculation of the biological units (BU) of the samples, the known BU of the reference were multiplied with the relative potency measured with the samples, which is the quotient of the 50% inhibition value of reference and sample. The statistical calculation of RAST inhibition was done as described by Anderson and Baer [20].

Results

SE-HPLC

Figure 1 shows the SE-HPLC traces of 4 runs out of 11, performed with the *D. pteronyssinus* extract on 2 days. All traces looked very similar, with almost

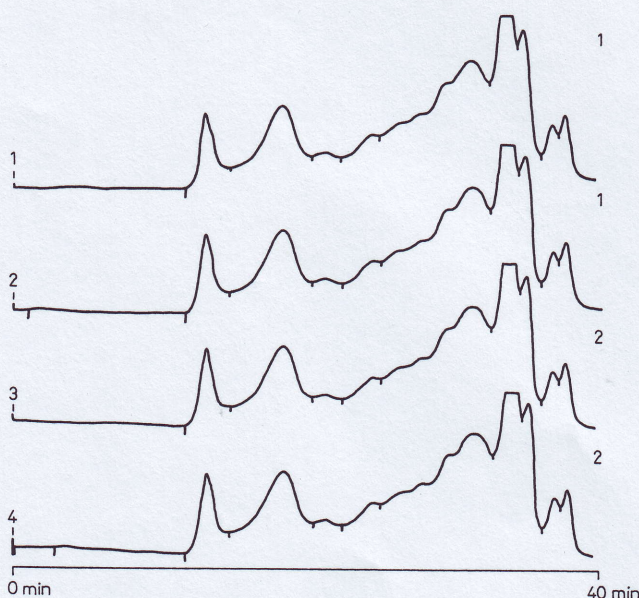


Fig. 1. SE-HPLC traces of 4 runs (numbered 1–4 on the left) of a *D. pteronyssinus* extract performed on 2 days (1, 2 on the right).

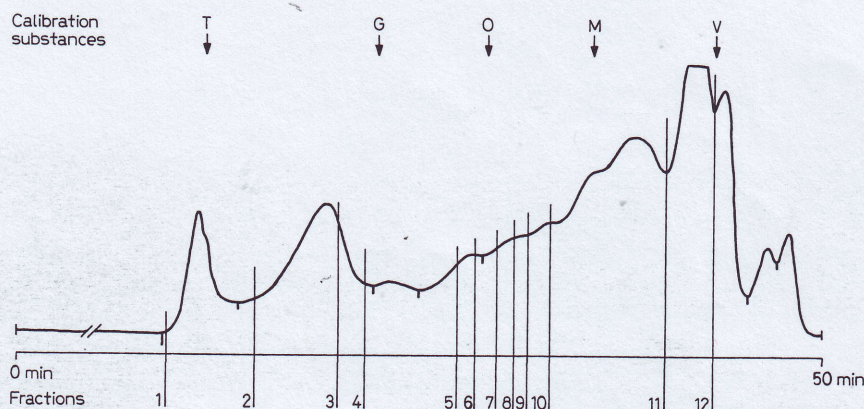


Fig. 2. Fractions isolated by SE-HPLC. The arrows indicate the retention times of the calibration substances: T = thyroglobulin (bovine; 670 kD); G = γ -globulin (bovine; 158 kD); O = ovalbumin (chicken; 44 kD); M = myoglobin (horse; 17 kD); V = vitamin B₁₂ (1.35 kD).

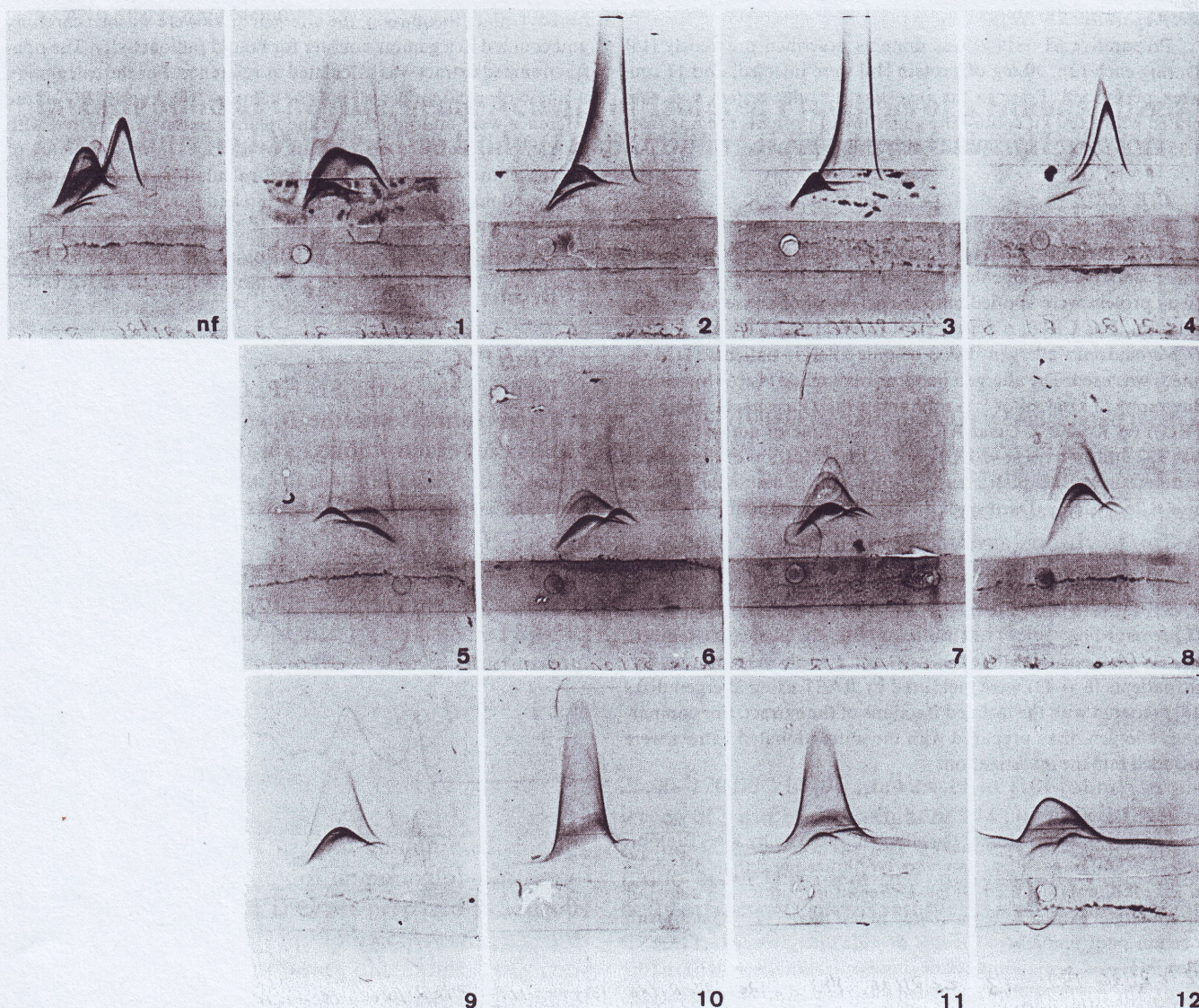


Fig. 3. CIE of the nonfractionated mite *D. pteronyssinus* extract (nf) and of fractions 1-12.

identical peak patterns, indicating a very good reproducibility of the employed method for SE-HPLC.

The fractions which were isolated by preparative SE-HPLC are indicated in figure 2. After isolation these fractions were lyophilized, and after reconstitution they were investigated by RAST, RAST inhibition and CIE/CRIE.

CIE/CRIE

The nonfractionated extract showed the most complex antigen/allergen patterns in CIE/CRIE. Most of the isolated fractions showed different precipitation lines in CIE (fig. 3).

Also by CRIE, most of the fractions showed differences in their allergen patterns, i.e. different quantities, different peak heights and radiostaining intensities of allergen bands (fig. 4). For example, fractions 10 and 11 consisted predominantly of the major allergen Der pI [21] and the cathodic migrating allergen PY [14].

RAST

RAST measurements of the sera of 8 mite-allergic patients done with the allergen disks prepared with fractions 1-12 and with the nonfractionated extract yielded binding rates between 5.1 and 24.8% (mean

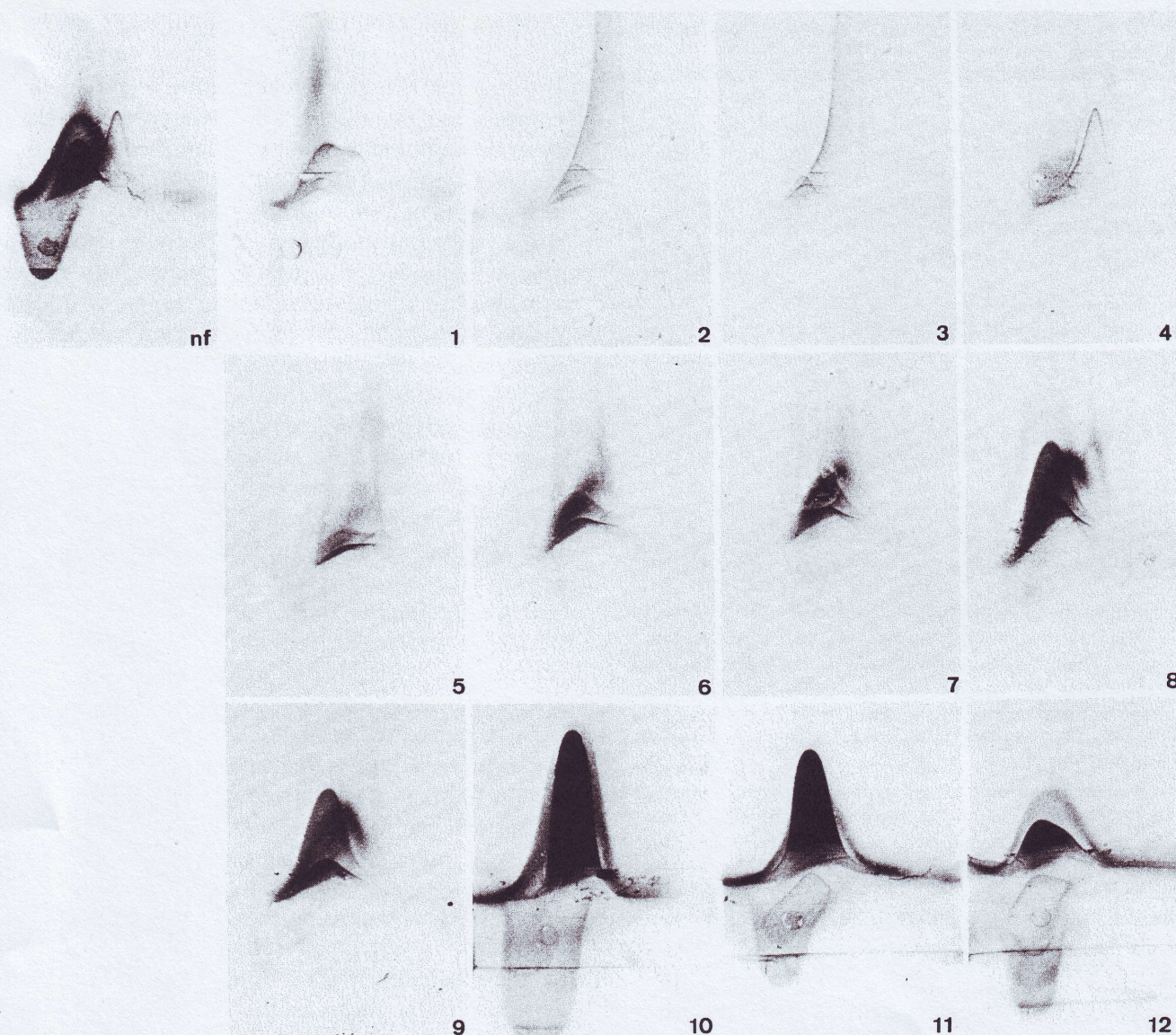


Fig. 4. CRIE of the nonfractionated mite *D. pteronyssinus* extract (nf) and of fractions 1-12. Time of autoradiography: 14 days.

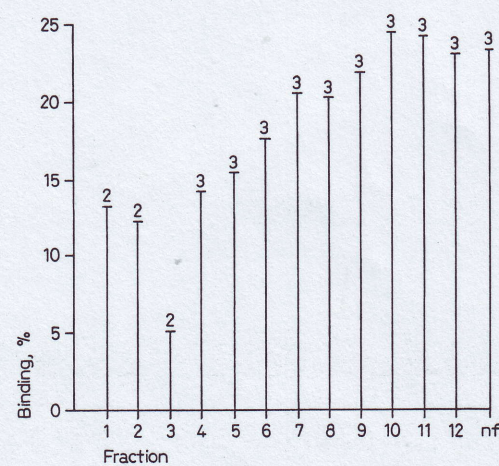


Fig. 5. RAST class measurements and percentage of bindings (mean values) of 8 sera of patients allergic to the mite *D. pteronyssinus* using allergen disks prepared with fractions 1-12 and with the nonfractionated extract (nf).

Table 1. RAST inhibition performed with fractions 1-12 and with the nonfractionated extract

Extracts	Protein $\mu\text{g/ml}$	Allergen BU/ml	Specific activity BU/ μg protein
Nonfractionated			
fractions	609	120,000	197
1	798	4,200	5.3
2	1,594	5,760	3.6
3	977	1,032	1.1
4	7,982	36,000	4.5
5	911	7,200	7.9
6	1,198	11,400	9.5
7	712	13,440	18.9
8	860	22,800	26.5
9	1,570	50,400	32.1
10	5,413	1,628,400	300.8
11	279	18,000	64.5
12	115	18,000	156.5

The nonfractionated extract was calculated as reference; BU = Biological units.

values). Allergen disks prepared with fraction 3 showed the lowest percentage of binding (5.1%). The highest binding rates, namely 23.2–24.8%, were measured with the allergen disks prepared with fractions 10–12. These binding rates were comparable to the one measured with the allergen disks prepared with the nonfractionated extract (23.8%) (fig. 5). With all allergen disks, RAST class 0 (0.44–0.83% binding) was measured with the nonallergic pool serum.

RAST Inhibition

The results of all RAST inhibition assays are summarized in table 1. According to the specific activity (BU/ μg protein), the fractions could roughly be separated into two groups. Fractions 7–12 showed high specific activities of 18.9–300.8 BU/ μg protein, whereas fractions 1–6 showed a lower specific activity, namely of 1.1–9.5 BU/ μg protein. The specific activity of fraction 10 was even 1.52 times higher than the one of the nonfractionated extract. The specific activity of fraction 3 was 179 times lower than the activity of the nonfractionated extract.

Discussion

Although allergen extracts show a heterogeneous composition of proteins and glycoproteins, in most cases the major allergens were identified in a molecular weight range of 10.00–60.00 kD [7]. Also in our investigations of a biologically standardized partly purified whole mite body extract (*D. pteronyssinus*) with several methods, the highest specific activity could be measured in a molecular weight range of approximately 2.00–18.00 kD. In this region we predominantly found the two major allergens Der pI [21] and PY [14]. In addition, our investigation showed also allergenic activity for fractions of higher molecular weights (> 158.00 kD). However, the specific activities of these fractions were lower than the ones of the fractions below 44.00 kD. By CRIE it could be shown that high molecular weight fractions contained allergens which were not present in the low/middle molecular weight fractions. This shows that also proteins of higher molecular weight have IgE-binding capacities. These IgE bindings were confirmed by RAST measurements of mite-allergic patients using allergen disks prepared from the isolated fractions.

Our findings are in agreement with published data from other authors, who also found allergens in regions of higher molecular weights [8–11]. In these cases the allergens were mostly of intermediate or minor character. According to a position paper on immunotherapy [12], an allergen extract should contain all allergens relevant for the patients, i.e. also intermediate and minor allergens.

The classification in major, intermediate and minor allergens is a statistical term. Therefore it is important to have a large amount of individual sera of allergic patients. The amount and quality of sera used for the classification has a great influence on the origin of the allergens.

Furthermore our results show that the method employed must be given when discussing molecular weight determination of allergens. In our investigation using the native *D. pteronyssinus* extract, the relative molecular weight of the major allergen Der pI could be roughly determined between 2.00 and 18.00 kD by SE-HPLC, whereas the literature describes a range of 10.00–30.00 kD [21] for the same allergen determined, however, by other methods.

In conclusion we could show that all isolated fractions including the fractions of higher molecular

weights from a biologically standardized partly purified whole mite body *D. pteronyssinus* allergen extract showed IgE-binding capacities and allergenic activities of different degrees.

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