

Biochemical and Allergenic Properties of the House Dust Mite Extract, *Dermatophagoides pteronyssinus*

AKIRA ISHII,¹ HIROSHI SHIMOMURA,¹ JUNICHI HASHIGUCHI² and YASUHIRO KABASAWA³

¹Dept. of Parasitology, Miyazaki Medical College, Kiyotake, Miyazaki,

²Dept. of Medical Biology, Institute of Basic Sciences, University of Tsukuba, Ibaraki, and

³Dept. of Pediatrics, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo, Japan

A further study has been made on the house dust mite extract, *Dermatophagoides pteronyssinus*, with emphasis on gel-filtrated fraction 2 (F₂). The crude mite extract showed at least nine discs on polyacrylamide disc electrophoresis and contained less than 0.25% sialic acid and less than 0.5 mM hexosamine and no detectable uronic acid. From gel filtration of the crude extract a No. 2 fraction (F₂) with allergenic activity showed at least five components on SDS disc electrophoresis covering a molecular weight range of between 15,000 and 70,000. The major allergenic activity of F₂ dissolved in pH 7-8 and 4-5 on an isoelectric focusing column. Affinity chromatography of lectins showed that allergenic activity did not relate to structures of *N*-acetyl-D-glucosamine or *N*-acetyl-D-galactosamine. Allergenic activity of the crude extract was not affected by peptic digestion and the mite digest prepared by trypsin and pronase showed a similar fractionation and activity profile as crude extract.

Key words: allergen; hypersensitivity; lectin; mite; RAST.

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House dust mite, genus *Dermatophagoides*, is the major constituent of the house dust allergen. According to Virchow et al. (21), about 90% of house dust atopy is caused by the mite. Our study of mite fauna in house dust collected from homes of asthmatic children showed that the average number of mites in 1 week was 135/m² and 75% of them were *Dermatophagoides pteronyssinus* and *D. farinae* (13). It has been reported that *D. pteronyssinus* is dominant in Europe and *D. farinae* is dominant in North America (22). In our study, although figures for *D. farinae* were slightly higher than for *D. pteronyssinus*, no significant statistical difference was found between them. Since *D. farinae* was the first to be successfully cultured in large quantities (19), biochemical and immunological studies have been made mainly on this species (10, 19).

Some analytical studies have clarified that there are many allergenic components in the mite extract, and Kabasawa & Ishii (15), using RAST inhibition with pooled sera from patients with mite allergy, proposed the practical usefulness of a No. 2 fraction (F₂) from gel-filtration of the crude extract in diagnosis and therapy. The F₂ was prepared as a biological product and supplied for clinical studies. In nasal allergy, it has been found that F₂ was efficacious for detecting cases with dust mite allergy (9). In the present report, we describe further the biochemical and allergenic characteristics of *D. pteronyssinus*, which is also an important species and has been successfully cultured by Miyamoto et al. (17), with special emphasis on F₂.

MATERIAL AND METHODS

The house dust mite

Dermatophagoides pteronyssinus was first isolated from a dust sample in 1972. It was cultured in Roux bottles according to the methods described by Miyamoto et al. (17). The saturated NaCl flotation method was used to collect the mites, after which they were stored in a Deep-freeze. The mite extract was prepared by homogenization and centrifugation as in our earlier study (11) with *D. farinae*.

Biochemical analysis

Protein concentration was estimated by ultraviolet absorption and/or the colorimetric method described by Lowry et al. (16) with BSA as a standard. Carbohydrate (reducing sugar) content was measured by the phenol-sulfuric acid method of Dubois et al. (7) with galactose as a standard. The content of sialic acid was estimated by the method of Aminoff (3) and *N*-acetyl-neuraminic acid from egg white (3× crystallized, Seikagaku kogyo, Tokyo) was used as a standard. Hexosamine was measured by the Elson-Morgan reaction modified by Good & Bessman (8) using D-glucosamine hydrochloride (Seikagaku kogyo, Tokyo) as a standard. Uronic acid was measured by carbazol-sulfuric acid reaction described by Dische (6) using D-glucuronic acid (Nakarai, Kyoto) as a standard.

Fractionation

Disc electrophoresis was carried out according to the methods described by Nagai (18). Amido black was used to stain protein components and the periodic acid Schiff reagent (PAS) was used to stain carbohydrates, as described by Segrest & Jackson (20). Gel-filtration was done by the use of Sephadex G-100 (Pharmacia, Sweden) and ion-exchange chromatography was performed using DEAE-Sephadex A50 by elution with a linear gradient concentration of NaCl, as described in our earlier studies (15).

Isoelectric focusing was carried out with a modified LKB 8101 apparatus. A density gra-

dient of 0–40% w/v sucrose containing carrier Ampholine (LKB, Sweden) covering a pH range 3–10 was prepared by manual overlaying of stepwise gradient concentrations.

Affinity chromatography was done by the use of Con-A Sepharose, wheat germ lectin Sepharose, *Helix pomatia* lectin Sepharose and lentil lectin Sepharose (Pharmacia, Sweden). Sample application was performed at a speed of 3.5 ml/h at 4°C and 2-ml fractions were collected. Bound material was further eluted out by the addition of the following reagents to the buffer; 0.1 M phosphate buffer to elute weakly bound glycoprotein and 0.1 M borate buffer to elute out remaining glycoprotein for Con A, 0.15 M methyl- α -D-glucoside for lentil lectin, 10 mg/ml *N*-acetyl-D-glucosamine for wheat germ lectin and 10 mg/ml *N*-acetyl-D-galactosamine for *Helix pomatia* lectin.

Allergen assay

Experimental animal system and radioallergosorbent test (RAST) were used to assess allergenic activity of the fractions obtained. Guinea pig sera sensitized by inhalation and/or injection with the mite extract were used in homologous Prausnitz-Küstner type skin reactions as reported in previous studies (10, 12). Sera of asthmatic children who showed positive immediate skin reaction to the mite were pooled and used in RAST. Cyanogen bromide activated paper discs (Toyo, Tokyo) were prepared by the methods of Wide et al. (24).

Enzyme treatments

Peptic digestion of the extract was performed using pepsin (32 mg, 3× crystallized, Nutritional biochem., U.S.A.) in 1 l 0.01 N HCl at pH 2. The mixture was incubated at 37°C overnight and the reaction stopped by adding 50% trichloroacetic acid 1:10. After centrifugation the supernatant was dialysed against saline before use. End-point dilution of the digest was examined to give a positive reaction larger than 10 mm diameter on guinea pig skin in passive cutaneous anaphylaxis (PCA).

Digestion of the crude extract with trypsin

and pronase was done in the same manner as described in the previous study with *D. farinae* (11). Fractionation of the digested extract was also performed as above using Sephadex G-100 and DEAE-Sephadex (Pharmacia, Sweden).

RESULTS

After 3 months of mass culture, an average of 2.18 g wet weight mite from 100 g powdered medium was collected on filter paper by the saturated NaCl flotation method as described by Miyamoto et al. (17). A rough estimate of the yield of mite extracts was 55 mg/g mite and approx. 30% carbohydrate content.

Sialic acid content was less than 8 µg/ml of the crude extract of which the protein content was 3.2 mg/ml. The crude mite extract contained less than 0.5 mM of both hexosamine and glucosamine. Uronic acid was not detected in the crude mite extract by the method employed.

The disc electrophoresis pattern of the crude extract is shown in Fig. 1 with rate of flow (Rf) values. At least nine distinct bands can be seen. PAS staining showed no distinct band and the starting point was only faintly stained. The mite extract digested with trypsin and pronase gave a lesser number of stained bands with Rf values 0.71, 0.79 and 0.93. SDS polyacrylamide electrophoresis was used to determine the molecular weights of the components of the allergenic gel-filtrated F₂ (Fig. 2). The molecular weights were between 15,000 and 70,000.

Isoelectric focusing of F₂ is shown in Fig. 3. The major allergenic portion was seen in pH 7.5.

Affinity chromatography on Con-A column is shown in Fig. 4. Relatively high activity was found in fractions eluted by 0.1 M phosphate buffer with low protein concentration. Fig. 5 shows the results on wheat germ lectin. No activity was detected in the adsorbed portion on *Helix pomatia* lectin or lentil lectin.

Allergenic activity of the mite extract was found in some degree resistant to enzymatic cleavage in our previous study (11). In the present study, in addition to trypsin and pronase, single treatment with pepsin was examined.

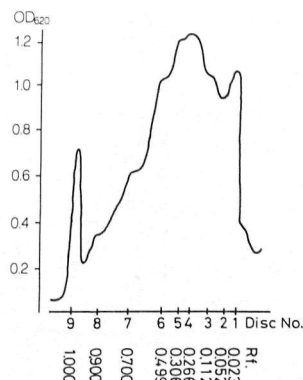


Fig. 1. Disc electrophoresis of the house dust mite, *Dermatophagoides pteronyssinus*.

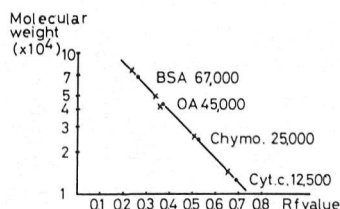


Fig. 2. Molecular weight of F₂ of the mite extract on SDS polyacrylamide gel electrophoresis. BSA: bovine serum albumin; OA: ovalbumin; Chy: chymotrypsin; Cyt.: cytochrome C; x: the stained bands.

Pepsin affected the allergenic activity of the crude mite extract at 1:63 dilution in the end-point of PCA but not as much as trypsin plus pronase did at 1:25 dilution.

Gel-filtration of the mite digest with trypsin and pronase on Sephadex G-100 gave a similar result to that obtained in the previous study with *D. farinae* (11). Allergenic activity was present in fractions of molecular weight ranging between 15,000 and 67,000. Pooled allergenic fractions were further applied on DEAE-Sephadex A50 column and the elution pattern was again similar to that obtained with *D. farinae* with guinea pig skin test activity. Allergens were eluted with NaCl of 0.5 M.

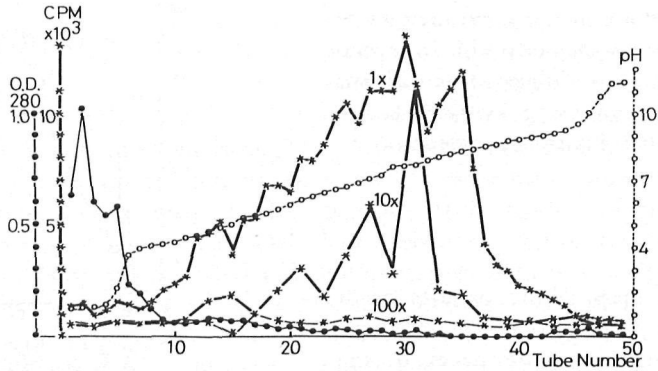


Fig. 3. Isoelectric focusing of F_2 of the mite extract (*Dermatophagoides pteronyssinus*) and allergenicity of the fractions as assayed by RAST.
1 $\times$, 10 $\times$, 100 $\times$ mean dilution power of the fractions.

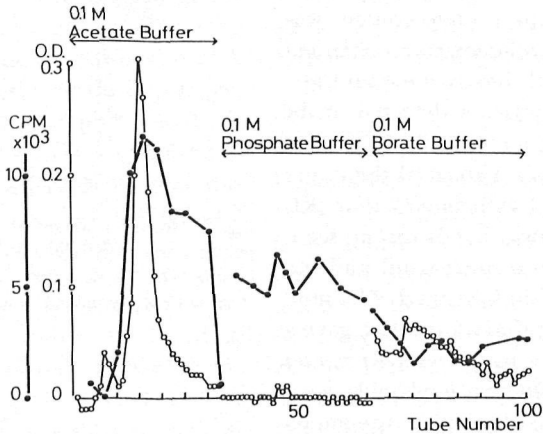


Fig. 4. Affinity chromatography of F_2 of the mite extract (*Dermatophagoides pteronyssinus*) on Con-A and allergenicity of the fractions as assayed by RAST.

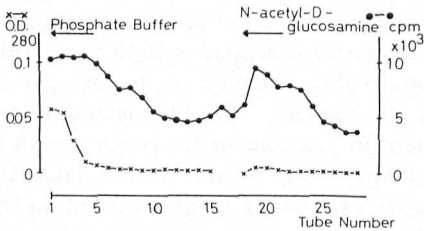


Fig. 5. Affinity chromatography of F_2 of the mite extract (*Dermatophagoides pteronyssinus*) on wheat germ lectin and allergenicity of the fractions as assayed by RAST.

DISCUSSION

Characterization of allergens has not been completed in many clinically important allergens because of many restricting factors, for example, the difficulty in making a biological assay of activity due to the ethical problem involved in provocation tests, polydispersity of the activity usually encountered in most fractionation studies, and difference in molecular

moiety between patient and animal models (2). Some of the common characteristics listed among allergens are: acidic glycoprotein with molecular weight range between 10,000 and 70,000, heat (boiling for 30 min) stable, and resistance to enzymic digestion, e.g. trypsin and pepsin (1). A number of studies have been made on the house dust mite allergen – the most important inhalant allergen in most countries – and Berrens (5) has published a monograph.

Since Voorhorst et al. (22) pointed out the importance of the mite contained in house dust, the clinical significance of the house dust mite allergen in non-seasonal atopic allergies has been confirmed in many parts of the world. Kabasawa et al. (14) reported the importance of *D. pteronyssinus* in asthmatic children in Japan and in a fractionation study obtained at least three fractions by column chromatographies (15). According to the results of RAST inhibition, most of the allergenic components are contained in the gel-filtrated fraction 2 which makes it clinically useful for diagnosis and hyposensitization.

The present study is a further characterization of the mite extract (*D. pteronyssinus*) with emphasis on F_2 . The results showed that the allergenic fraction 2 is composed of more than five disc bands with molecular weights ranging between 15,000 and 70,000. Isoelectric focusing showed allergenicity to be mainly in neutral and acidic fractions. Disc electrophoresis of the crude extract, *D. pteronyssinus*, showed nine countable disc bands which is fewer than the number in *D. farinae* (19). However, the difference in densitometric patterns is not as conspicuous as with other species. Although few specific components have been identified for both *D. farinae* and *D. pteronyssinus*, shared antigenic components were also shown by gel diffusion and immunoelectrophoresis (25).

In *D. pteronyssinus* content of sialic acid was less than 0.25% whereas in *D. farinae* it was less than 0.4% (11). According to a monograph by Berrens (5), the hexosamine content of house dust allergens was between 0–5.6% and uronic acid was positive up to 20%. In this study uronic acid was not detected.

Baldo et al. (4) reported the use of the lectin Tridacnin, for the isolation of allergen from house dust mite (*D. farinae*), and discussed its usefulness in the standardization of allergen preparations. The specific binding characteristics of the lectin from the clam, *Tridacna maxima* are not clear. In the present study, we examined some available lectins to clarify the nature of allergenic components, if at all possible. According to the results with Con-A, weakly bound glycoprotein was allergenic with low figures of OD₂₈₀ and high scores in RAST. Mannosyl or glucosyl residues might have certain relations to allergenic components. On the other hand, neither *N*-acetyl-D-glucosamine nor *N*-acetyl-D-galactosamine showed any relevance to allergenic structures. If any more lectins with different structural specificity became available it might be possible to analyse the allergenic determinants. But there is at least the possibility to utilize the available lectins for fractionation of allergens from the crude extract.

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Address:

A. Ishii
Dept. of Parasitology
Miyazaki Medical College
Kiyotake
Miyazaki, 889-16
Japan

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