

# Purification and Characterization of a Major Allergen from the House Dust Mite *Dermatophagoides farinae*

A. HOLCK<sup>1</sup>, S. DALE<sup>2</sup> and K. SLETTEN<sup>3</sup>

<sup>1</sup>Dept. of Biochemistry, University of Bergen, <sup>2</sup>New Venture R & D. Nycomed AS, Oslo, and <sup>3</sup>Dept. of Biochemistry, University of Oslo, Norway

A major allergen from an extract of the house dust mite, *Dermatophagoides farinae*, was shown to be extremely heterogenic with respect to charge. A slightly basic component of this allergen with a pI of 8, was purified by isoelectric focusing in two steps. The purified component, denoted antigen 19/20 IIa, seemed to be representative for the allergenic activity of the major allergen. The amino acid analysis suggested that antigen 19/20 IIa had a molecular weight of 9400 and contained one residue of galactosamine. SDS polyacrylamide gel electrophoresis did indicate a somewhat higher molecular weight of 14,500. Antibodies against the purified component cross-reacted with a crude extract of the mite *Dermatophagoides pteronyssinus*.

**Key words:** allergens; crossed radioimmunoelectrophoresis; *Dermatophagoides farinae*.

Accepted for publication 11 March 1986

The pyroglyphid mites in house dust are identified as the main source of house dust allergens and are responsible for allergic asthma and rhinitis in many patients (23, 27, 32).

Allergen extracts find wide application in the diagnosis and immunotherapy of immediate allergy. It is therefore essential that the extracts are well characterized and contain sufficient amounts of the allergens which are relevant for the patients in question.

Many different allergens have been obtained in a highly purified form. These allergens have been used as standards for the allergen content of extracts and have also been widely used in research.

Allergenic fractions from the house dust mite *Dermatophagoides farinae* have been isolated and characterized (18, 19, 24, 29). One of the allergens has been purified to homogeneity (10).

The allergens of a crude *D. farinae* extract have been identified by means of crossed radioimmunoelectrophoresis (CRIE) using individual patient sera (9). Specific IgE was frequently found to three of the mite antigens. Con-

sequently, these antigens were classified as major allergens. One of the major allergens formed a complex immunoprecipitate giving the impression of antigenic heterogeneity. This antigen was denoted antigen 19/20. Specific IgE to antigen 19/20 was demonstrated in 22 of 25 mite allergic patient sera, a frequency which was exceeded by only one other antigen.

The aims of the present work were to purify and characterize antigen 19/20. The purification was performed with a partially purified and commercially available allergen preparation, Spectralgen Mite *D. farinae*. This preparation is described in a previous paper (9).

The purified allergen was studied by means of CRIE and radioallergen sorbent test (RAST)-based techniques and the *in vivo* activity confirmed by skin prick tests (SPT).

## MATERIAL AND METHODS

*D. farinae* and *Dermatophagoides pteronyssinus* extracts were provided by Nycomed A/S, Nor-

way. *D. farinae* crude extract and the purified preparation denoted Spectralgen Mite *D. farinae*, were prepared as described previously (9). The crude extract from *D. pteronyssinus* was prepared essentially as the *D. farinae* crude extract.

Rabbit antisera against the crude mite extract of *D. farinae* was prepared as described previously (9). Rabbit antisera against purified allergen was prepared essentially as described by Harboe & Ingild (14). Two rabbits were immunized and boosted monthly with 30 µg of purified allergen. The rabbits were bled every 4 weeks, starting 2 weeks after the fourth injection.

Patient serum A. U. was kindly provided by Dr. Jacob Havnen, Rogaland Central Hospital, Norway.

#### *Crossed immunoelectrophoresis*

CIE was performed on glass slides (5 × 7 cm) essentially as described by Weeke (34) using a 1% agarose gel (Litex, Glostrup, Denmark) containing a buffer of 0.073 M Tris, 0.024 M barbital, 0.006 M Ca-lactate and 0.003 M sodium-azide (pH 8.6, 20°C). The first and second dimension electrophoreses were run at 12°C at 10 V/cm for 35 min, and at 18°C at 2 V/cm for 20 h respectively. The slides were pressed, washed, dried and stained with Coomassie Brilliant Blue. Heating of the slides to be used in CRIE was avoided. Such slides were stored dry at 5°C and not stained until after completion of the autoradiography.

#### *Crossed radioimmunoelectrophoresis*

CRIE was performed essentially as described by Löwenstein et al. (21). The CIE preparations were incubated overnight with 0.4 ml patient serum in incubation buffer I: 0.0025 M sodium phosphate buffer pH 7.4, 0.2% (w/v) tween 20, 0.9% NaCl to a final volume of 7 ml. After washing four times with incubation buffer I, the slides were incubated overnight at 20°C with <sup>125</sup>I-anti-IgE (Phadebas RAST isotope) solution, approximately 250,000 cpm in 7 ml of incubation buffer II: 0.05 M sodium phosphate buffer pH

7.4, 0.15 M NaCl, 0.3% HSA, 0.05% sodium-azide, 0.5% tween 20. After washing four times with incubation buffer I, and once with distilled water, the slides were dried and laid face down on X-Omat X-ray films (Kodak) for autoradiography. Exposure times varied from 1–6 weeks. The same procedure was also employed for the slide obtained by isoelectric focusing in the first dimension gel followed by immunoelectrophoresis in the second dimension (see below).

#### *Isoelectric focusing in polyacrylamide gel followed by immunoelectrophoresis*

IEF was performed with preformed Ampholine-PAG-plates (pH 3.5–9.5) as described by the producer (LKB, Bromma, Sweden). Ribonuclease A, haemoglobin and ovalbumin were used as reference proteins. To follow the focusing, bromphenol blue was added to the reference proteins. After focusing, the PAG-plate was laid face down on an agarose gel with antibody-containing gels on both sides. The antigens were then transferred to the antibody-containing gels by overnight electrophoresis at 2 V/cm 18°C. Autoradiography of the slide was performed as described above.

#### *Tandem crossed immunoelectrophoresis*

Tandem CIE was performed as described by Krøll (17). Antigens were applied in two wells 1 cm apart for the first dimension electrophoresis. The experimental conditions were as described for CIE, except that 1st dimension electrophoresis was reduced to 25 min.

#### *Double immunodiffusion*

The technique of Ouchterlony was followed (25). The experiment was performed on a glass slide (5 × 7 cm) using a 1.5 mm layer of 1% agarose gel as in CIE. Six peripheral wells were cut around a central well so that the distance between each well was 12 mm. The diffusion took place overnight in a humid chamber at room temperature and in horizontal position. The slide was pressed, washed, dried and stained as in CIE.

### *RAST inhibition*

Twenty  $\mu$ l serum and 30  $\mu$ l allergen extract (1.75 g/l protein) were incubated overnight in individual test tubes before addition of allergosorbent. The allergosorbent was Spectralgen Mite *D. farinae* (1.0 g/l dry weight) coupled to cyanogen bromide-activated paper discs according to Ceska et al. (7). RAST was performed as recommended by Pharmacia. The results were expressed as uptake of  $^{125}$ I-anti-IgE (Phadebas RAST isotope, Pharmacia Diagnostics AB, Sweden) in per cent of the total applied or expressed as per cent inhibition. The RAST results, obtained with 20  $\mu$ l serum and 30  $\mu$ l buffer and with only 50  $\mu$ l buffer, were used in the calculations as the 0% and 100% inhibition value, respectively. In terms of cpm the 100% inhibition value was insignificantly lower than that obtained with the inhibited serum (Table 2).

### *Skin prick test*

SPT was performed according to the procedure described by Aas et al. (1). Eight patients selected for hyposensibilization with Spectralgen *D. farinae* were tested in duplicate with purified antigen 19/20 IIa (60  $\mu$ g/ml protein) and Spectralgen *D. farinae*, 10,000 biological units (BU) and 50,000 BU. The Spectralgen *D. farinae* solutions contained 15  $\mu$ g/ml and 75  $\mu$ g/ml protein, respectively. The skin reaction was calculated by means of  $(D + d)/2$ , where D is the largest diameter and d is the diameter perpendicular to D. The mean skin reaction of the duplicates was calculated.

### *Preparative flat-bed isoelectric focusing*

Preparative isoelectric focusing in a granulated gel was performed essentially as described by the producer (LKB, application note 198) with a prewashed Ultradex gel. The ampholyte concentration was kept 2–4% (initial concentration). After focusing, a protein print was made from the gel which was subsequently divided and eluted with cold distilled water to measure the pH. The gel was then eluted with buffer to redissolve possibly precipitated proteins. The

protein print was scanned with a Zeineh soft-laser scanning densitometer, Biomed Instruments Inc., USA, and the data recorded on Hewlett Packard integrator 3390 A. Assuming that all the protein bands were coloured equally well, it was possible to obtain a measure of the relative amounts of protein in each printed band.

Protein was determined by a modification of the method of Lowry et al. (20) as described by Bensadoun & Weinstein (5).

### *Polyacrylamide gel electrophoresis*

Discontinuous polyacrylamide gel electrophoresis in acidic environment was performed essentially after the method of Davis (11), employing 7.5% polyacrylamide in the separating gels with a K-acetate buffer pH 4.5. Polyacrylamide gel electrophoresis in basic environment was performed in 8% polyacrylamide gels in a 0.375 M Tris-sulphate buffer pH 9.0, the reservoir buffer being 0.065 M Tris-borate pH 9.0. The samples were made 0.07 M Tris-sulphate and 10% glycerol prior to electrophoresis.

### *Estimation of molecular weight*

Sodium dodecyl sulphate electrophoresis was performed essentially as described by Weber & Osborne (33) in 0.1 M sodium phosphate buffer pH 7.2, 0.1% SDS, 0.1%  $\beta$ -mercaptoethanol in 10% polyacrylamide gels. The samples were mixed to 0.2 M sodium phosphate pH 7.2, 1% SDS, 1%  $\beta$ -mercaptoethanol, 20% glycerol and incubated at 60°C for 1 h prior to electrophoresis. Aprotinin (6500), cytochrome C (12,500) and trypsin inhibitor (21,000) were used as reference proteins. The gels were fixed by incubating in 12% TCA for 1 h, stained with Coomassie Brilliant Blue and destained until the background was clear. Scanning of the gels was performed at 600 nm with a DU spectrophotometer model 2400, Beckmann Instruments, USA, with a Gilford gelscanner, Gilford, USA.

Automatic amino acid analysis were performed essentially as described by Sletten et al. (30).

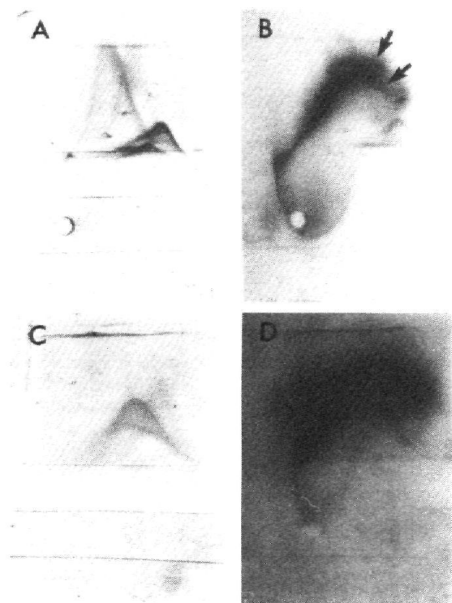


Fig. 1. Crossed radioimmuno-electrophoresis pattern of mite extracts. (A) The CIE pattern of crude mite extract, 90  $\mu$ g applied. (B) Autoradiography of the same slide after incubating with patient serum A.U. The arrows pinpoint the double precipitate. (C) The CIE pattern of Spectralgen Mite *D. farinae*, 10  $\mu$ g applied. (D) Autoradiography of C with patient serum A.U. The presence of a small radiostaining precipitate in B was due to unspecific binding of antigen 19/20 (9).

The protein sample was hydrolysed for 24 h with 6 M HCl in evacuated tubes at 110°C.

*N-terminal analysis.* Dansylation was performed on 130  $\mu$ g of antigen 19/20 IIa and separation of the dansyl amino acids was as described by Ambler & Brown (2) and Gray (13).

## EXPERIMENTS AND RESULTS

Crossed radio immuno-electrophoresis with a crude extract of *D. farinae* showed that patient serum A. U. reacted only towards antigen 19/20, and not to any other of the identified allergens in the crude extract (Fig. 1A, B). The serum was consequently useful as a tracer

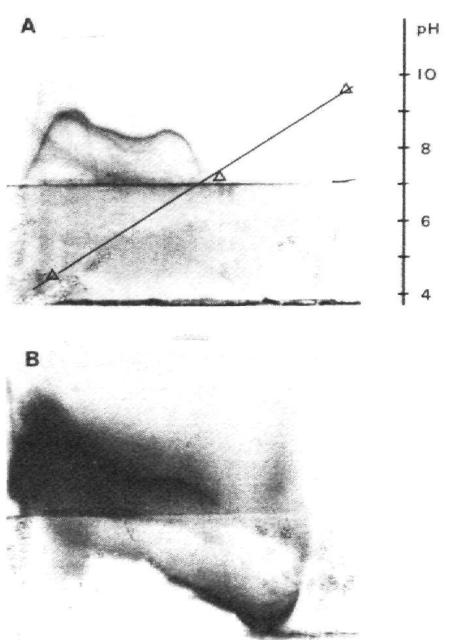


Fig. 2. Analytic isoelectric focusing of Spectralgen Mite *D. farinae* followed by immuno-electrophoresis into an antibody-containing gel, 40  $\mu$ g applied. (A) The antigen pattern after staining the slide with Coomassie Brilliant Blue. (B) Autoradiography of the same slide after incubating with patient serum A.U.  $\Delta$  - pI reference proteins: chicken ovalbumin pI = 4.6, rat haemoglobin pI = 7.0-7.4, ribonuclease *Streptococcus* pI = 9.5.

serum during the present work. CRIE with Spectralgen Mite *D. farinae* confirmed the presence of antigen 19/20 in this partly purified preparation (Fig. 1C, D).

The CRIE pattern revealed the heterogeneity of this antigen. Sometimes two distinct anodic precipitates were seen (hence the double numbering 19/20) as in Fig. 1B. A cathodic precipitate was also observed. The precipitin line of this cathodic component was confluent with the anodic precipitate. The upper part of the anodic precipitate sometimes gave rise to a broad and diffuse radiostaining.

Analytic isoelectric focusing of the Spectralgen Mite *D. farinae* was followed by immu-

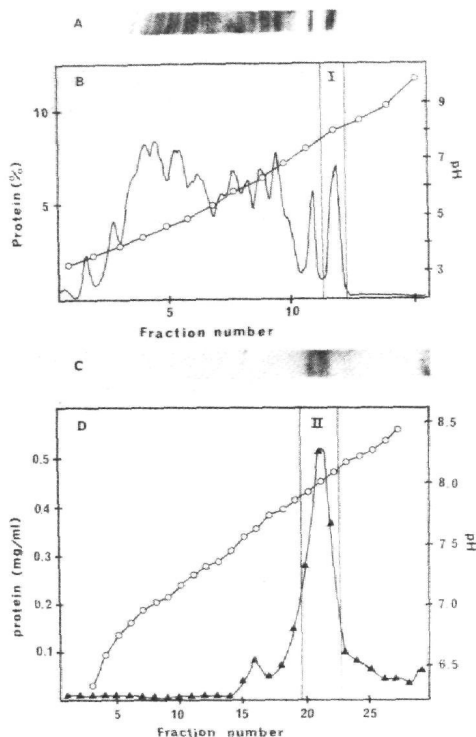


Fig. 3. Purification of antigen 19/20 IIa by isoelectric focusing in two steps. (A) The protein print from IEF of Spectralgen Mite *D. farinae* in granulated gel pH 3.5 to 9.5. (B) Scan of A demonstrating Fraction I. Protein is in % of total. (C) The protein print from IEF pH 7 to 9 of Fraction I. (D) Protein measured in the fractions from IEF pH 7 to 9 and demonstration of Fraction II. O - pH measurements.

noelectrophoresis into an antibody-containing gel (Fig. 2). Autoradiography of the slide with patient serum A. U. confirmed the heterogeneity of antigen 19/20 and showed several components with isoelectric pI ranging from about 4.0–8.0. The other antigens focused largely in the pI range 3.5–7.0.

The slightly basic component giving rise to the cathodic precipitate was isolated by preparative isoelectric focusing in granulated gel.

Spectralgen Mite *D. farinae* was first subjected to isoelectric focusing using a wide pH gradient ranging from 3.5–9.5. A protein print taken

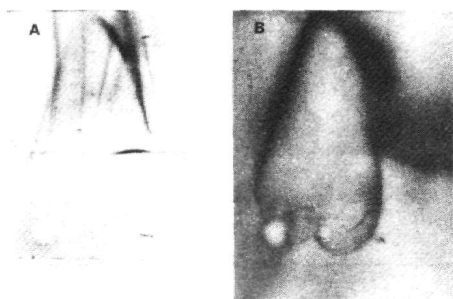


Fig. 4. Tandem crossed radioimmuno-electrophoresis of the purified antigen 19/20 IIa together with Spectralgen Mite *D. farinae*. (A) CIE pattern, anodic well: 1 µg Spectralgen Mite *D. farinae* cathodic well: 40 µg Spectralgen Mite *D. farinae*. (B) Autoradiography of the same slide after incubating with patient serum A.U. The horizontal radiostaining spur was caused by capillary forces during the first dimensional electrophoresis (6).

from the gel bed demonstrated a protein focusing at pH 8.0 (Fig. 3A). From a scan the protein at pH 8.0 seemed to comprise 7% of the printed protein (Fraction I, Fig. 3B).

Fraction I was then collected and refocused with a narrow gradient of pH 7–9. The material focused in two bands with pI 7.93 and 8.01 (Fig. 3C) while the protein elution pattern showed only one peak between pH 7.8 and pH 8.0 (Fig. 3D). The peak fractions were pooled (Fraction II), concentrated by ultrafiltration and gelfiltered through Sephadex G-25 to remove ampholytes. The purified antigen was denoted antigen 19/20 IIa. The recovery after the purification is summarized in Table 1.

The identity of antigen 19/20 IIa was con-

Table 1

Purification scheme of antigen 19/20 IIa. The dry material and protein in some fractions from the purification steps

| Fraction                              | Dry material<br>mg | Protein<br>mg |
|---------------------------------------|--------------------|---------------|
| Spectralgen mite<br><i>D. farinae</i> | 897 (100%)         | 395 (100%)    |
| IEF, Fraction I                       | —                  | —             |
| IEF, Fraction II                      | —                  | 8             |
| Isolated                              | 14 (1.6%)          | 7 (1.8%)      |
| antigen 19/20 IIa                     |                    |               |

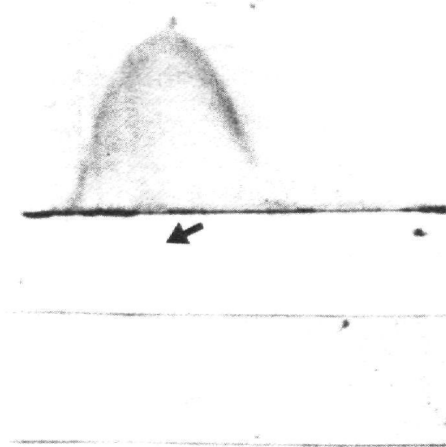


Fig. 5. Crosed immunoelectrophoresis of purified antigen 19/20 IIa (17.5  $\mu$ g). Arrow shows the position of a minor contamination.

firmed by tandem-CRIE, showing a continuous precipitate at the cathodic side (Fig. 4). The purified antigen 19/20 IIa also formed precipitates at the anodic side of the slide and showed identity with the acidic components of antigen 19/20.

The identity was also confirmed by RAST-inhibition. The RAST reaction of patient

Table 2

Comparison of antigen 19/20 IIa and Spectralgen Mite *D. farinae*. A RAST-inhibition experiment using a tracer serum for antigen 19/20 IIa (patient serum A.U.) and a Spectralgen Mite *D. farinae* allergosorbent

| Inhibitor                             | Counts in %<br>of total | % inhibition |
|---------------------------------------|-------------------------|--------------|
| Spectralgen Mite<br><i>D. farinae</i> | 1.2                     | 90           |
| Antigen 19/20 IIa                     | 1.1                     | 93           |
| Buffer                                | 16.8                    | 0            |

serum A.U. with Spectralgen Mite *D. farinae* was inhibited to the same degree by antigen 19/20 IIa and the Spectralgen preparation (Table 2). This indicates that this basic component is representative for the allergenic activities of the antigen 19/20 in the Spectralgen Mite *D. farinae*, and that this *in vitro* activity is retained throughout the purification procedure. The *in vitro* activity was further confirmed by the observation that the antigen still bound IgE in CRIE.

The *in vivo* activity of the purified antigen was investigated by skin prick tests on eight *D. farinae* mite-allergic patients. All eight patients reacted towards the purified antigen 19/20 IIa. The results are presented in Table 3 and confirm the high *in vivo* activity and the importance of this allergen.

The immunological purity of antigen 19/20 IIa was investigated in a CIE experiment with large amounts of the purified antigen (17.5  $\mu$ g) against anti-crude *D. farinae* antiserum. The CIE pattern revealed a minor contamination as indicated by the arrow in Fig. 5. It is noticeable that antigen 19/20 IIa gave rise to an anodic precipitate in this experiment.

The purity of the antigen 19/20 IIa preparation was investigated by polyacrylamide gel electrophoresis in acidic and basic environment (Fig. 6). In the acidic electrophoresis, antigen 19/20 IIa appeared as one major band with a minor lagging contamination which made up about 7% of the protein as estimated from the

Table 3

Mean skin reactions of eight Spectralgen *D. farinae*-sensitive patients towards the purified antigen 19/20 IIa

| Patient | Antigen<br>19/20 IIa | Spectralgen mite, <i>D. farinae</i> |           |
|---------|----------------------|-------------------------------------|-----------|
|         |                      | 10,000 BU                           | 50,000 BU |
| P.Ch.   | 8.6                  | 8.1                                 | -*        |
| A.F.    | 11.9                 | 7.8                                 | -         |
| O.L.    | 7.8                  | 5.3                                 | -         |
| K.H.    | 7.4                  | 7.1                                 | -         |
| S.A.S.  | 7.0                  | 5.1                                 | 7.6       |
| B.G.    | 10.5                 | 7.0                                 | 9.3       |
| J.Aa.   | 12.0                 | 6.0                                 | 9.0       |
| O.M.    | 11.8                 | 8.5                                 | 10.0      |

\* Not determined.

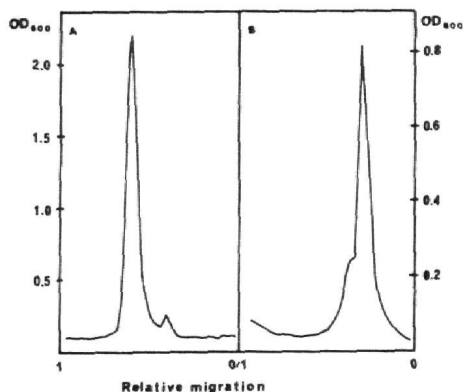


Fig. 6. Polyacrylamide gel electrophoresis of antigen 19/20 IIa. (A) 50  $\mu$ g antigen was electrophoresed in Tris-borate buffer pH 9, migration was relative to bromphenol blue. (B) 9  $\mu$ g antigen was electrophoresed in a K-acetate buffer pH 4.5, migration was relative to methyl green. The gels were coloured with Coomassie brilliant blue and scanned at 600 nm. In both cases the electrophoresis was from right to left.

scanning area. In the basic electrophoresis, antigen 19/20 IIa appeared as one major band with a minor leading contamination which made up about 15% of the protein.

The molecular weight of antigen 19/20 IIa was estimated by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis. The antigen gave rise to one band with an apparent molecular mass of 14,500 (Fig. 7).

The amino acid composition of antigen 19/20 IIa is given in Table 4. Based on one residue of methionine the antigen 19/20 IIa protein was found to consist of 84 amino acid residues and one residue of galactosamine. This corresponds to a molecular weight of about 9400. This value is somewhat different from that obtained from SDS polyacrylamide gel electrophoretic measurements. Gelfiltration of antigen 19/20 IIa on a column of Sephadex G-50 superfine gave a molecular mass of about 8500 (results not shown).

The N-terminal amino acid of antigen 19/20 IIa was investigated by the reaction of dansyl-chloride. No dansyl-amino acid derivative was, however, found, indicating that the antigen has a blocked N-terminal.

Antibodies against antigen 19/20 IIa were raised in rabbits. The antibodies were shown to be monospecific in a CIE/CRIE experiment using crude *D. farinae* extract and a patient serum pool (results not shown). These monospecific antibodies cross-reacted with an antigen from a crude extract of the mite *D. pteronyssinus* (Fig. 8).

## DISCUSSION

The analytic isoelectric focusing experiment and the CIE/CRIE experiments showed that antigen 19/20 was extremely heterogenic with respect to charge. The antigen was also found to contain a component which focused with a slightly basic pI. The variations in pI might be explained by differences in amide content or carbohydrate content of the protein, although other factors, such as conformational changes, might be involved (28). The presence of "iso-allergens", i. e. proteins with different pIs but with the same allergenic activity, is quite com-

Table 4

Amino acid composition of antigen 19/20 IIa. 280  $\mu$ g of the purified antigen was hydrolysed *in vacuo* in 6 N HCl for 24 h at 110°C and analysed on a BioCal BC 200 amino acid analyser. The total number is based on one residue of methionine

| Amino acid and amino sugar | Composition residues/molecule |
|----------------------------|-------------------------------|
| Asx                        | 14.0                          |
| Thr                        | 5.4                           |
| Ser                        | 2.5                           |
| Glx                        | 7.0                           |
| Pro                        | 4.3                           |
| Gly                        | 5.4                           |
| Ala                        | 8.2                           |
| Cys                        | 2.8                           |
| Val                        | 6.3                           |
| Met                        | 1.0                           |
| Ile                        | 6.0                           |
| Leu                        | 5.0                           |
| Tyr                        | 1.3                           |
| Phe                        | 2.8                           |
| His                        | 2.7                           |
| Lys                        | 8.8                           |
| Arg                        | 1.1                           |
| GalN                       | 0.75                          |

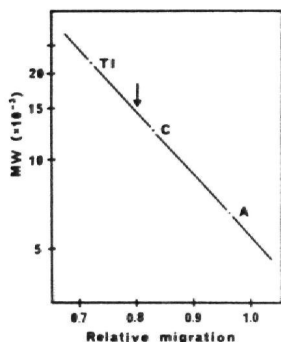


Fig. 7. Sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis of antigen 19/20 IIa. Migration was relative to bromphenol blue. TI = trypsin inhibitor (21,000), C = cytochrome C (12,500), A = aprotinin (6500). Arrow indicates the position of purified antigen 19/20 IIa.

mon and has also been reported in extracts from another species of house dust mite. Chapman & Platt-Mills (8) have shown that the  $P_1$  antigen from *D. pteronyssinus* gave two bands of different pI upon isoelectric focusing. Extracts from many sources, including rye (22), cod (12) and *Cladosporium herbarum* (3), have also been shown to contain isoallergens. It is not known whether these isoallergens are present *in vivo* or are due to the purification procedure.

Purification by isoelectric focusing in a flat-bed was technically easy and allowed the preparation of paper prints showing the protein distribution. The vast majority of the proteins, including the acidic components of antigen 19/20, focused with pIs in the range of pI 3.5–7.0. This enabled a high degree of purification of the slightly basic component antigen 19/20 IIa.

Identification of the isolated component as a variant of antigen 19/20 was performed by tandem-CRIE. The use of autoradiography was essential in this respect because of the weak staining of the antigen in CIE. The identity was also verified by RAST inhibition. The results from the RAST inhibition experiment indicated that the observed molecular variants of antigen 19/20 were identical with respect to antigenicity in one human IgE system.

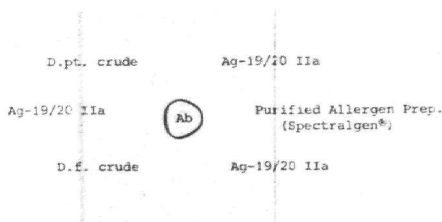


Fig. 8. Double immunodiffusion showing the cross reactivity of antibodies against an antigen from *D. pteronyssinus*. The central well contains monospecific antibodies against antigen 19/20 IIa, 20  $\mu$ l. Purified antigen 19/20 IIa, 7  $\mu$ g, Spectralgen Mite *D. farinae*, 20  $\mu$ g, and crude extracts from *D. farinae* and *D. pteronyssinus*, 90 and 130  $\mu$ g respectively, were applied in the other wells.

It has been shown that the faecal balls from the genus *Dermatophagoides* have allergenic activity (31). However, antigen 19/20 does not seem to be associated with these faecal balls (15).

The immunological purity of antigen 19/20 was investigated by CIE against anti-crude mite antiserum. The slide confirmed the purity in that only a minor contaminant was detected. By this technique it is always a possibility that other contaminants may escape detection, which happens if the corresponding antibody is not present in the antiserum. The purity was thus further investigated by polyacrylamide gel electrophoresis at pH 4.5 and pH 9.0. From these studies the antigen was judged to be at least 85% pure.

The purified antigen 19/20 IIa with a pI of 8.0 would normally be expected to migrate slowly towards the anode in the first dimension electrophoresis in crossed immunoelectrophoresis and to form anodic precipitates in the antibody-containing gel in the second dimension electrophoresis. This was the case when large amounts of protein were applied to the gel (Fig. 5). However, frequently a precipitate formed at the cathodic side of the slide. This was probably due to electroendosmotic flow in the agarose gel, a phenomenon which is observed especially when the pI of the antigen is close to the pH of the electrophoresis (4).

The molecular mass of antigen 19/20 IIa falls within the range of about 5000 to 70,000 which is the usual limit for allergens from *D. farinae*, as determined by several authors (9, 16). The molecular weight obtained by gelfiltration (8500) was somewhat different from that obtained by SDS-PAGE (14,500). Similar discrepancies have been reported earlier, for example in the case of the histones which by SDS-PAGE do not follow the usual standard protein curve (26). Comparison of the molecular weight obtained by gelfiltration with the apparent molecular weight obtained by SDS-PAGE, however, suggested that antigen 19/20 IIa could be considered as a single polypeptide chain. Taking into account the data from the amino acid analysis, antigen 19/20 IIa would be a single chain glycoprotein containing one residue of galactosamine. However, the total carbohydrate composition of antigen 19/20 IIa was not obtained. It is an open question whether the carbohydrate residue is important for the allergenic activity.

Antigen 19/20 IIa is not identical to the allergen Ag-11 of Dandeu et al. (10). This is evident from measurements of the molecular weight by SDS polyacrylamide gel electrophoresis and data obtained by amino acid analysis, differing, for example, with respect to Gly and Glx content and Lys/Arg ratio.

The close phylogenetic relationship between different species of house dust mites is confirmed by the finding that antibodies against antigen 19/20 IIa cross-reacted with a crude extract of the mite *D. pteronyssinus*. Cross-reactivity between antigens from *D. farinae* and *D. pteronyssinus* has also been reported by others (19).

## ACKNOWLEDGEMENT

We wish to thank Dr. Arve B. Frostad, Allergologisk Poliklinikk, Oslo, for performing the skin prick tests.

## REFERENCES

1. Aas, K., Backmann, A., Belin, L. & Weeke, B.: Standardization of allergen extracts with appropriate methods. *Allergy* 33, 130-137, 1978.
2. Ambler, R. P. & Brown, S. M.: The amino acid sequence of *Pseudomonas fluorescens* azurin. *Biochem. J.* 104, 784-825, 1967.
3. Aukrust, L. & Borch, S. M.: Partial purification and characterization of two *Cladosporium herbarum* allergens. *Int. Arch. Allergy Appl. Immunol.* 60, 68-79, 1979.
4. Axelsen, N. H., Bock, E., Larsen, P., et al.: Immunoprecipitation in an electric field. *Scand. J. Immunol.* 17, Suppl. 10, 87-96, 1983.
5. Bensadoun, A. & Weinstein, D.: Assay of proteins in the presence of interfering materials. *Anal. Biochem.* 70, 241-250, 1976.
6. Bjerrum, O. J.: Electroimmunoprecipitation artefacts. *Scand. J. Immunol.* 17, Suppl. 10, 333-342, 1983.
7. Ceska, M. & Lundkvist, U.: A new and simple radioimmunoassay method for the determination of IgE. *Immunochemistry* 9, 1021-1030, 1972.
8. Chapman, M. D. & Platt-Mills, T. A. E.: Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P<sub>1</sub>. *J. Immunol.* 125, 587-592, 1980.
9. Dale, S. & Landmark, E.: Characterization of allergens in a crude extract of *Dermatophagoides farinae* and their identification in a new purified preparation. *Allergy* 39, 572-585, 1984.
10. Dandeu, J.-P., Le Mao, J., Lux, M., Rabillon, J. & David, B.: Antigens and allergens in *Dermatophagoides farinae* mite II. Purification of AG 11, a major allergen in *Dermatophagoides farinae*. *Immunology* 46, 679-687, 1982.
11. Davis, B. S.: Disc electrophoresis II. Methods and applications to human serum proteins. *Science* 121, 404, 1964.
12. Elsayed, S. & Aas, K.: Isolation of purified allergens (cod) by isoelectric focusing. *Int. Arch. Allergy Appl. Immunol.* 40, 428-438, 1971.
13. Gray, W. R.: End-group analysis using dansyl chloride. *Methods Enzymol.* 25, 121-138, 1972.
14. Harboe, N. & Ingild, A.: Immunization, isolation of immunoglobulins, estimation of antibody titre. *Scand. J. Immunol.* 2, Suppl. 1, 161-164, 1973.
15. Holck, A. L. & Dale, S.: Allergens of the house dust mite *D. farinae*. Presented at the XI International Congress of Allergy & Clinical Immunology, London, 1982.
16. Krilis, S., Baldo, A. B. & Basten, A.: Dialyzable allergens from the common house dust mites. *Naturwissen.* 66, 475-476, 1979.
17. Krøll, J.: Tandem crossed immunoelectrophoresis. *Scand. J. Immunol.* 2, Suppl. 1, 57-60, 1973.
18. Le Mao, J., Dandeu, J.-P., Rabillon, J., Lux, M. & David, B.: Antigens and allergens in *Dermatophagoides farinae* mite. I. Immunochemical and physicochemical study of two allergenic fractions from a partially purified *Dermatophagoides farinae* mite extract. *Immunology* 44, 239-247, 1981.
19. Le Mao, J., Dandeu, J.-P., Rabillon, J., Lux, M. & David, B.: Comparison of antigenic and allergenic composition of two partially purified extracts from *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* mite cultures. *J. Allergy Clin. Immunol.* 71, 588-596, 1983.

20. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R. J.: Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265-275, 1951.
21. Løwenstein, H., Aukrust, L. & Gravesen, S.: *Cladosporium herbarum* extract characterized by means of quantitative immunoelectrophoretic methods with special attention to immediate type allergy. *Int. Arch. Allergy Appl. Immunol.* **55**, 1-12, 1977.
22. March, D. G.: Allergens and the genetics of allergy. In: Sela, M. (ed.): *The antigens*, Vol III. Academic Press, New York. 271-359, 1976.
23. Maunsell, K., Wraith, D. G. & Cunningham, A. M.: Mites and house-dust allergy in bronchial asthma. *Lancet* **i**, 1267-1270, 1968.
24. Nakagawa, T., Kudo, K., Okudaira, H., Miyamoto, T. & Horiuchi, Y.: Characterization of the allergenic components of the house dust mite *Dermatophagoides farinae*. *Int. Arch. Allergy Appl. Immunol.* **55**, 47-53, 1977.
25. Ouchterlony, O.: Diffusion-in-gel methods for immunological analysis. *Progr. Allergy* **5**, 1-78, 1958.
26. Panyim, S. & Chalkley, R.: The molecular weights of vertebrate histones exploiting a modified sodium dodecyl sulphate electrophoretic method. *J. Biol. Chem.* **246**, 7557-7560, 1971.
27. Pauli, G., Bessot, J. C., Guisard, G., Roegel, E. & Oudet, P.: Importance clinique des acariens chez les asthmatiques allergiques à la poussière domestique. *Rev. fr. Allergy* **12**, 141-153, 1972.
28. Righetti, P. G. & Drysdale, J. W.: Isoelectric focusing. In: Work, T. S. & Work, E. (eds.): *Laboratory techniques in biochemistry and molecular biology*. Vol. 5. North Holland Publishing, Amsterdam, 527-585, 1976.
29. Romagnani, S., Biliotti, G., Passaleva, A. & Ricci, M.: Mites and house dust allergy. II. Relationship between house dust and mite (*Dermatophagoides pteromyssinus* and *Dermatophagoides farinae*) allergens by fractionation methods. *Clin. Allergy* **2**, 115-123, 1972.
30. Sletten, K., Natvig, J. B., Husby, G. & Juul, J.: The complete amino acid sequence of a prototype immunoglobulin- $\lambda$  light-chain-type amyloid fibril protein AR. *Biochem. J.* **195**, 561-572, 1981.
31. Tovey, E. R., Chapman, M. D. & Platt-Mills, T. A. E.: Mite faeces are a major source of house dust allergens. *Nature* **289**, 592-593, 1981.
32. Voorhorst, R., Spieksma-Boezema, M. I. A. & Spieksma, F. Th. M.: Is a mite (*Dermatophagoides sp*) the producer of house dust allergen? *Allergie Asthma* **10**, 329-334, 1964.
33. Weber, K. & Osborn, M.: The reliability of molecular weight determinations by dodecyl sulphate polyacrylamide gel electrophoresis. *Biol. Chem.* **244**, 4406-4412, 1969.
34. Weeke, B.: Quantitative immunoelectrophoresis. General remarks on principles, equipment, reagents and procedures. *Scand. J. Immunol.* **2**, Suppl. 1, 15-35, 1973.

Address:  
S. Dale  
New Venture R & D  
Nycomed AS  
Nycoveien 2  
Oslo 4  
Norway

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.