

Characterization of a Very High Density Lipoprotein Allergen, Dpt 4, from the House Dust Mite *Dermatophagoides pteronyssinus*

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Abstract. The allergen Dpt 4 from the house dust mite *Dermatophagoides pteronyssinus* was shown to be a lipoprotein as judged by its Sudan black B staining properties. Ultracentrifugal studies confirmed this finding and showed that Dpt 4 was polydisperse, although the majority of the allergen belonged to a very high density lipoprotein class. Physicochemical studies using gradient polyacrylamide gel electrophoresis showed that Dpt 4 had an apparent molecular weight of 244,000 daltons and a particle diameter of 10.73 nm. Agarose gel electrophoresis showed that the allergen had an electrophoretic mobility 0.86 times that of human α -lipoprotein. The observation that Dpt 4 is a lipoprotein is consistent with earlier studies which showed that the allergen was heterogeneous with regard to its molecular weight and that it contained the hapten phosphorylcholine.

Introduction

Studies from this laboratory have shown that extracts of the house dust mite, *Dermatophagoides pteronyssinus*, contain an allergen designated Dpt 4 [19, 20] which exhibits certain physicochemical characteristics differing markedly from those associated with previously described allergens from other sources. For example, by gradient polyacrylamide gel electrophoresis the bulk of the allergen was found to have an apparent molecular weight of 274,000 daltons, a value outside the commonly accepted molecular weight range of 10,000-60,000 daltons circumscribing the allergens from grass pollens and danders [13]. In addition, Dpt 4 demonstrated heterogeneity with regard to both molecular weight and binding to the lectin Con-A [19, 20]. Further, this allergen was shown to contain the hapten phosphorylcholine (PC) as judged by its reactivity with the PC-specific murine IgA myeloma S107 [20, 21].

These observations have prompted further studies not only to determine whether Dpt 4 represents a major or minor allergen in an allergic population but also to determine its precise biochemical structure. In this regard, experiments aimed at elucidating the structure of Dpt 4 have been performed and the following report details some of the experimental results

obtained. Such data show that the allergen Dpt 4 is a very high density lipoprotein thus accounting for both the observed molecular weight and carbohydrate heterogeneity and the presence of the hapten PC.

Materials and Methods

Preparation of Mite Extracts

House dust mite, *D. pteronyssinus*, and the media upon which it was grown were kindly provided by Commonwealth Serum Laboratories (Parkville, Vict.) and extracted as described by Stewart and Turner [19]. Extracts were dialyzed against distilled water and, after freeze drying, stored over desiccant at -20°C.

Electrophoretic Methods

Crossed immunoelectrophoresis (CIE) and immunoelectrophoresis (IEP) were performed as described previously [18, 19] using anti-whole mite extract antisera. CIE and IEP plates were stained for protein using Biebrich scarlet [7] and for lipoprotein using Sudan black B [23].

Agarose gel electrophoresis was performed using 1.2% agarose (Type 5, Sigma Chemical Co.) in barbitone buffer, pH 8.6 (1 = 0.05). Samples were prestained with Sudan black B as described by Narayan [14] and electrophoresed at 2 V/cm for 2 h at 4°C. Gels were photographed immediately after completion of electrophoresis.

Gradient polyacrylamide gel electrophoresis (GPAGE) was performed at 20°C using the Pharmacia gel electrophoresis apparatus (GE-4) (South Seas Pty.) and prepacked gradient gels (PAA 4/30). Electrophoresis was performed according to the manufacturers' instructions. Molecular weight (MW) markers were pur-

Lipoprotein Mite Allergen

chased from Pharmacia (South Seas Pty.) and included thyroglobulin (MW 669,000), ferritin (MW 440,000), catalase (MW 232,000), lactate dehydrogenase (MW 140,000) and albumin (MW 67,000). Molecular weight determinations were performed at least three times and the data were analyzed by linear regression.

The particle diameter (in nm) of Dpt 4 was determined in a manner similar to that described earlier [2, 4, 15] using the above molecular weight reference proteins. The particle diameters of the above proteins used in calculations were 17.0, 12.2, 10.4, 8.16, and 7.1 nm, respectively. The data were analyzed by linear regression analysis.

Samples were prestained for lipoprotein with Sudan black B [14] and photographed immediately after electrophoresis. Gels were subsequently stained for protein with Coomassie blue G250 [3].

Detection of Phosphorylcholine in Mite Extracts

The presence of PC in mite fractions was determined using an inhibition of hemagglutination assay employing the PC-specific mouse myeloma S107 and sheep red blood cells coated with an extract of *Ascaris suum* peritene fluid as described earlier [20]. Results were expressed in hemagglutination inhibition units (HAIU), where one unit represented the volume of test substance required to inhibit the agglutination of sensitized cells by the S107 myeloma.

Preparative Ultracentrifugation

All ultracentrifugation experiments were performed in a Beckman Model L ultracentrifuge at 14°C using a Ti 50 rotor employing experimental conditions commonly used in the isolation of human lipoprotein classes. Whilst the relationship between mite and human lipoprotein classes is unknown, the terminology used to describe the latter has been adopted throughout this study. Low density lipoproteins (LDL) were isolated at 140,000 g for 24 h using a NaCl-KBr solvent of density 1.063 g/ml [8]. At the end of the run the top 2-ml fraction from each centrifuge tube was removed and the density of the remaining solution adjusted to a density of 1.210 g/ml by the addition of solid KBr. High density lipoproteins (HDL) were isolated at this density by centrifugation at 140,000 g for 40 h [1] after which the three consecutive 2-ml fractions from each tube (designated fractions 1, 2, and 3, respectively) were removed. Very high density lipoprotein (VHDL) separations were performed at a solvent density of 1.250 g/ml at 165,000 g for 44 h [1]. Two consecutive 1-ml fractions (designated fractions 1 and 2, respectively) from each tube were removed and the remaining fraction (fraction 3) concentrated by ultrafiltration using a SM 13200 colloidal bag (Sartorius GmbH, Göttingen). All ultracentrifuge fractions were dialyzed against 0.15 M NaCl containing 0.01 M EDTA for 48 h at 4°C. The solvent density ranges of the lipoprotein-containing fractions were determined by reference to an appropriate standard curve of density versus volume constructed as described by Lindgren et al. [11]. All density measurements were performed at 20°C using a Paar Mettler DMA 35 digital density meter [10].

Results

GPAGE Studies

An extract of whole mite was prestained with the lipophilic stain Sudan black B and electrophoresed

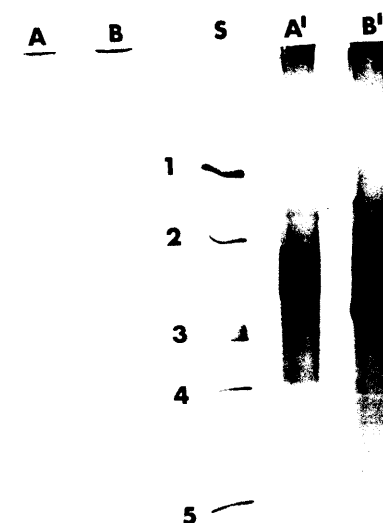


Fig. 1. Gradient (4-30%) polyacrylamide gel electrophoretic analysis of unspent growth medium (A, 55 μ g) and whole mite extract (B, 55 μ g) prestained for lipid with Sudan black B and postelectrophoretically stained for protein with Coomassie blue G250 (A', B'). The molecular weight markers (S) were: 1 = thyroglobulin (MW 669,000); 2 = ferritin (MW 440,000); 3 = catalase (MW 232,000); 4 = lactate dehydrogenase (MW 140,000) and 5 = albumin (MW 67,000).

on a 4-30% gradient polyacrylamide gel. After electrophoresis, the gels were examined for the presence of lipid staining material and then stained for protein with Coomassie blue G250. Control experiments using an extract of growth medium were also included.

Typical results from such GPAGE experiments are shown in figure 1. Only one lipid-staining band was detected in the whole mite extract (B). In contrast, this band was not seen in the unspent growth medium extract (A). Subsequent staining for protein (A', B') indicated that the lipid-staining material also stained for protein. The apparent molecular weight and particle diameter of this lipoprotein were 244,000 daltons and 10.73 nm, respectively.

CIE Studies

Extracts of whole mite were examined by CIE for lipoprotein-containing precipitin bands by staining

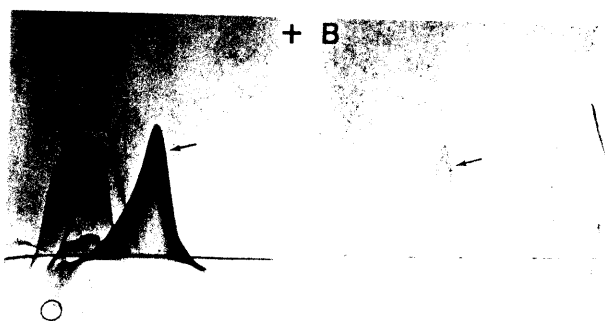


Fig. 2. Crossed immunoelectrophoretic analysis (CIE) of *D. pteronyssinus* stained for protein with Biebrich scarlet (A) and for lipid with Sudan black B (B) using 200 and 100 μ g of extract, respectively. CIE was performed at an antibody concentration of 43 μ l per cm^2 gel area. The arrow indicates the allergen Dpt 4.

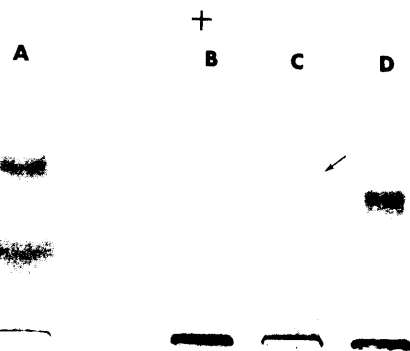


Fig. 3. 1.2% agarose gel electrophoretic analysis of (A) human serum (6 μ l), (B) unspent growth medium (66 μ g), (C) spent growth medium (6 μ g), and (D) whole mite extract (66 μ g) after prestaining for lipid with Sudan black B. The arrow indicates the position of a brown band associated with an unknown component in the spent mite medium extract rather than a lipid-staining component.

CIE gels with Sudan black B. The results from these experiments are shown in figure 2. Again, only one lipid-containing component was identified as indicated by the arrow (B). The precipitin band corresponded to the characteristically densely protein-staining band associated with the allergen Dpt 4 (A) described earlier [19, 20].

Agarose gel Electrophoresis

The electrophoretic mobility of the mite lipoprotein was compared with that of the mobilities of human α - and β -lipoprotein by agarose gel electrophore-

sis. Typical results from such experiments are shown in figure 3. Such studies showed that the mite lipoprotein had a relative electrophoretic mobility 0.86 times that of human α -lipoprotein. The lipoprotein was not detected in either unused growth medium or in used growth medium (devoid of whole mites) by this assay. However, electrophoretically diffuse lipid-staining material was seen in both these extracts. In addition, densely lipid-staining material was seen at the origin in all three mite extracts to varying degrees.

Ultracentrifugal Analysis

Extracts of whole mite were subjected to preparative ultracentrifugation at three solvent densities, namely, 1.063, 1.210, and 1.250 g/ml. After ultracentrifugation the fractions were examined by IEP analysis using an anti-whole mite extract antiserum. IEP studies failed to detect the mite lipoprotein in the top fraction from material centrifuged at a solvent density of 1.063 g/ml (data not shown). However, the lipoprotein was detected in the top fractions from material centrifuged in solvent at both 1.210 and 1.250 g/ml. Analysis of the two remaining fractions from the 1.210 g/ml solvent run indicated that the mite lipoprotein was dispersed throughout the tube and, in addition, at a much greater concentration than in the top fraction as judged empirically by the size of the precipitin band after IEP analysis (data not shown). Fractions 1 and 2 from the 1.250 g/ml solvent run also contained the mite lipoprotein (fig. 4) but at a concentration greater than that seen in fraction 1 from the 1.210 g/ml solvent run using the above criterion. Other mite components were detected in fractions 1 and 2 from the 1.250 g/ml solvent run after staining

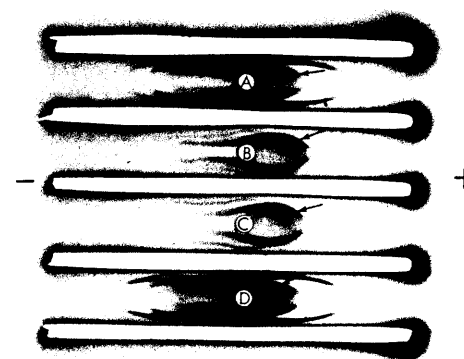


Fig. 4. Immunoelectrophoretic analysis of various fractions from a preparative ultracentrifugal separation of an extract of *D. pteronyssinus* at a density of 1.25 g/ml performed in a Beckman Ti 50 rotor at 42,000 rpm for 42 h. A represents the original material, B represents fraction 1 (density range 1.200–1.205 g/ml), C represents fraction 2 (density range 1.206–1.213 g/ml) and D represents fraction 3 (density > 1.213). The antiserum used was an anti-whole mite extract antiserum. The plate was initially stained for lipoproteins with Sudan black B and then stained for protein with Biebrich scarlet. The allergen Dpt 4 is indicated with an arrow.

for protein (fig. 4) but unlike Dpt 4 they did not stain for lipid (data not shown).

The density range of fraction 1 from the 1.210 g/ml run was 1.160–1.175 g/ml and the density ranges of fractions 1 and 2 from the 1.250 g/ml solvent run were 1.200–1.205 and 1.206–1.213 g/ml, respectively.

The PC content of the fractions obtained from the 1.250 g/ml solvent run was determined using a hemagglutination-inhibition assay. These studies showed that the majority of the PC activity was present in fraction 3 (table I). However, PC activity was also found in association with fractions 1 and 2; fractions which had specific activities of 640 and 320 HAIU/ml solution, respectively.

Discussion

The allergen Dpt 4 has been judged to be a lipoprotein on the basis of its ultracentrifugal floatation and Sudan black B staining properties. However, the paucity of material has precluded an extension of this study to determine its relationship to other well de-

Table I. Reactivity of various *Dermatophagoides pteronyssinus* fractions isolated by ultracentrifugation in a solvent of density 1.250 g/ml with the phosphorylcholine-specific murine myeloma S107 in a hemagglutination inhibition assay

Fraction	Density range g/ml	Reactivity with S107		
		Specific activity in HAIU/ml	Total activity	Recovery %
Original extract	-	5,120	53,760	-
Fraction 1	1.200–1.205	640	640	1.1
Fraction 2	1.206–1.213	320	320	0.6
Fraction 3	> 1.213	15,360	53,760	100

Results are expressed in HAIU/ml, where 1 hemagglutination inhibition unit represents the minimum volume of inhibitor required to inhibit the agglutination of sensitized cells by the S107 myeloma.

scribed lipoproteins from humans and experimental animals on the basis of its percentage cholesterol, tri-glyceride and phospholipid composition [6]. Despite this, however, these data together with data generated in previous studies [19, 20] are consistent with the suggestion that Dpt 4 is a lipoprotein since similar properties have been ascribed to lipoproteins from humans [reviewed in 5].

GPAGE studies showed that Dpt 4 had a particle diameter of 10.73 nm (corresponding to an apparent molecular weight of 244,000 daltons); a value in between those described for the human HDL subclasses 2a and 2b which had particle diameters of 10.1 and 11.1 nm, respectively [15]. The apparent molecular weight of Dpt 4 determined in this study is slightly lower than that previously reported. The reason for this is not known but may reflect the use of different starting materials.

Preparative ultracentrifugation of Dpt 4 showed that it was polydisperse with regard to density, although such studies did show that it belonged primarily to the very high density class. Here again, Dpt 4 displayed polydispersity in that it was distributed between the top and bottom ultracentrifugation fractions, a situation analogous to that described for human VLDL [1].

Earlier studies from this laboratory [20] showed that allergen Dpt 4 contained the hapten PC. Analysis of the ultracentrifuge fractions described in this study

are consistent with these studies. However, the majority of the PC hapten was found in fraction 3 (density > 1.213). Similar findings have been reported for human VHDl where it was shown that the bottom VHDl fraction, designated VHDl2, comprised 75–85% of the total VHDl phospholipid [1, 22]. Although Dpt 4 is present in fraction 3, it is not yet known whether all the PC contained therein is associated with Dpt 4 or whether it is found in association with other molecules such as albumin; a situation which has been described with human and rat sera [1, 22]. Gel filtration studies [20] did show, however, that PC was not associated with a carrier mite molecule analogous to albumin in terms of molecular weight since all of the PC activity was associated with high molecular weight components (> 70,000 daltons).

Throughout this and earlier studies the presence of the hapten PC has been used as a marker for the allergen Dpt 4 without knowing whether such a marker indicated that Dpt 4 belonged to one of several classes of compounds known to contain similar moieties such as C-substances (from bacteria and fungi) and lipoproteins [9, 12, 17]. Data from this study indicate that Dpt 4 is a lipoprotein, thus suggesting that it contains not PC per se but PC containing phospholipids such as phosphatidylcholine or lysophosphatidylcholine; phospholipids which are known to be present in lipoproteins.

These phospholipids have not yet been chemically identified within Dpt 4, but evidence exists which shows that assays analogous to the one used to detect PC in this and previous studies will detect lipoproteins per se [9]. In addition, it has been shown that a mouse myeloma idiotypically related to S107 will recognize only these phospholipids containing the PC moiety [16]. Studies from this laboratory [unpublished observations] have shown that the hemagglutination inhibition assay used in these studies will recognize both β -lipoproteins and phosphatidylcholine.

In summary, the allergen Dpt 4 from the house dust mite *D. pteronyssinus* has been shown to be a VHDl with an electrophoretic mobility slightly slower than that of human α -lipoprotein. It had a particle diameter of 10.73 nm and an apparent molecular weight of 244,000 daltons. Such findings, together with unpublished observations from this laboratory which show that lipoproteins from parasites represent extremely potent allergens, suggest that lipoproteins are worthy of further study in regard to immediate hypersensitivity.

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