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Physicochemical Characterization of a Major Protein Allergen, *Der p I*, from the House Dust Mite, *Dermatophagoides pteronyssinus*

Amino Acid Analysis and Circular Dichroism Studies

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Abstract. A major house dust mite allergen, *Der p I*, was isolated from spent growth medium and physicochemically characterized. These studies show that the allergen is monomeric, contains approximately 216 residues and 4 intra-chain disulphide bonds. The N-terminal amino acid is threonine. Circular dichroism studies show that the allergen contains 10% α -helical, 50% β -pleated sheet and 40% random structures.

Introduction

Pre-eminent amongst allergens involved in precipitating allergic diseases such as asthma and rhinitis are those derived from the house dust mite, *Dermatophagoides pteronyssinus* [1, 2], and because of this, many studies have been devoted to the isolation and characterization of mite allergens. In particular, such studies have been undertaken to gain insight into the factors influencing the aetiology (i.e., initial sensitization and provocation of symptoms) of mite-induced allergic disease, to develop standardized diagnostic reagents and, ultimately, to optimize immunotherapeutic procedures.

However, although several groups have shown that mite extracts contain several major and minor allergens [3-5], the number of mite allergens that have been purified is limited. This, together with the paucity of structural data on individual mite allergens at the molecular level, has retarded or even precluded the achievement of some of these goals. To this end, we present a physicochemical study of a major mite allergen, *Der p I*, and, in particular, describe its amino acid composition and secondary structural characteristics as determined by circular dichroism (CD) analysis.

Materials and Methods

Der p I was isolated from spent mite growth medium using a combination of hydrophobic interaction chromatography, gel filtration and chromatofocusing as described previously [6]. The allergen was further purified, first by fast liquid protein chromatography on a TSK 3000 SW column and then by reverse-phase high-performance liquid chromatography. The allergen was then reduced and alkylated using dithiothreitol and iodoacetic acid as described previously [7]. The S-carboxymethylated allergen was purified by gel filtration and subjected to amino acid analysis and N-terminal amino acid analysis [7].

CD studies were performed on a Cary 61 recording spectropolarimeter as previously described [8]. A mean residue weight of 110 was assumed in all calculations and the CD measurements were expressed as the mean residue ellipticity in $\text{deg} \cdot \text{cm}^2/\text{dmol}$. The amounts of α helix, β -pleated sheet and random structures were determined by the method of Greenfield and Fasman [9].

Results

A detailed amino acid analysis of the allergen was performed, and the results are shown in table I together with data derived from Chapman and Platts-Mills [10]. The data are presented as residues per 100 residues and as residues per molecule. In the latter, a carbohydrate-free molecular weight of 24,732 daltons

was used, based upon the sodium dodecylsulphate polyacrylamide gel electrophoretically derived apparent molecular weight, namely 27,000 daltons [6] and 8.4% carbohydrate [10]. In general, there was good agreement between the amino acid content, expressed as residues per 100 residues, determined by Chapman and Platts-Mills [10] and that determined in this study. Certain small quantitative differences were, however, evident, particularly with regard to the content of serine, glycine, isoleucine and half-cystine. It is probable that these differences reflect the hydrolysis conditions employed, namely a single time point hydrolysis [10] versus a time course series of hydrolyses (this study). The N-terminal amino acid was shown to be threonine.

The CD spectrum of *Der p* I in the region of 200 to 240 nm is shown in figure 1. The spectrum was characterized by two negative CD maxima at 216 nm and 208 nm, both with mean residue ellipticity values of 6,150 deg · dmol. The secondary structure content of *Der p* I was derived from this spectrum and the allergen was shown to contain approximately 10% α helix, 50% β -pleated sheet structure and 40% random structure.

Discussion

Limited structural studies from this and other groups [11] showed that the allergen *Der p* I [previously described as P₁, Dpt 12 and Dp 42, ref. 11] is a monomeric glycoprotein with an apparent molecular weight of 25,000–30,000 daltons. Thus, the structural studies described here further extend our knowledge of the molecular characteristics of the allergen. In particular, the data presented indicated that this allergen comprised approximately 216 amino acid residues with 4 intra-chain disulphide bonds and that threonine was the N-terminal amino acid. In addition, circular dichroism studies showed that the allergen contained very little α -helical structures in contrast to high levels of β -pleated sheet and random structures.

Further studies are underway to determine the complete amino acid sequence of this allergen and to initiate epitope-mapping studies in an attempt to define those regions of the molecule involved in triggering allergic disease. Such epitopes will then be synthesised and their use as immunotherapeutic reagents assessed.

Table I. Amino acid composition of *Der p* I

Amino acid	Residues/100 residues		Residues per molecule Dpt 12
	P ₁ ^a	Dpt 12 ^b	
Asx	13.9	13.0	28
Thr	4.4	3.8	8
Ser	7.8	4.7	10
Glx	12.9	11.2	24
Pro	5.2	4.7	10
Gly	10.3	8.2	17
Ala	9.3	8.7	19
1/2 Cys	1.0	3.7 ^c	8
Val	5.2	5.5	12
Met	1.2	1.5	3
Ile	6.2	7.8	17
Leu	4.4	4.4	9
Tyr	5.4	6.2	13
Phe	2.2	1.7	4
His	2.6	2.9	6
Lys	2.1	1.3	3
Trp	ND	6.0 ^d	13
Arg	5.6	5.8	12

The molecular weight was assumed to be 24,732 daltons; see text. P₁, Dpt 12 = Respective authors' names for *Der p* I; ND = not determined.

^a Data from Chapman and Platts-Mills [10], derived from 24-hour hydrolysis of fraction 14.

^b Time course hydrolysis.

^c Half-cystine determined as S-(carboxymethyl)-cysteine.

^d Obtained after methane sulphonic acid hydrolysis.

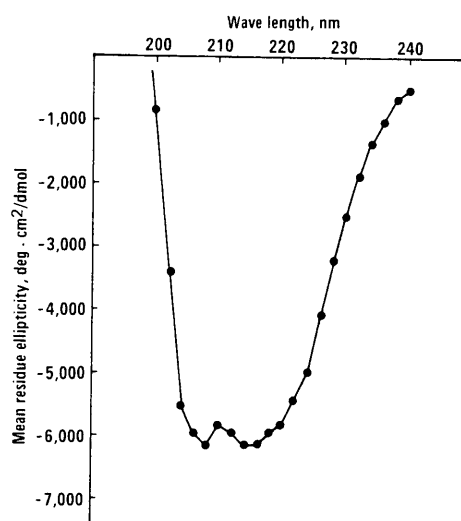


Fig. 1. CD spectrum of *Der p* I in the region of 200 to 240 nm, showing two negative maxima at 208 and 216 nm.

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References

- 1 Voorhorst, R.; Spieksma-Boezeman, M.I.A.; Spieksma, F.T.M.: Is a mite (*Dermatophagoides* sp.) the producer of the house dust allergen? *Allergie Asthma* 10: 329 (1964).
- 2 Maunsell, K.; Wraith, D.G.; Cunningham, A.M.: Mites and house dust allergy in bronchial asthma. *Lancet* i: 1267 (1968).
- 3 Stewart, G.A.; Butcher, A.; Lees, K.; Ackland, J.: Immunochemical and enzymatic analyses of *Dermatophagoides pteronyssinus*. *J. Allergy clin. Immunol.* 77: 14-24 (1986).
- 4 Lind, P.; Lowenstein, H.: Identification of allergens in *Dermatophagoides pteronyssinus* mite body extract by crossed radioimmunoelectrophoresis with two different rabbit antibody pools. *Scand. J. Immunol.* 17: 263-273 (1983).
- 5 Krilis, S.; Baldo, B.A.; Basten, A.: Antigens and allergens from the common house dust mite *Dermatophagoides pteronyssinus*. II. Identification of the major IgE binding antigens by crossed radioimmunoelectrophoresis. *J. Allergy clin. Immunol.* 74: 142-146 (1984).
- 6 Stewart, G.A.: The isolation and characterization of the allergen Dpt 12 from *Dermatophagoides pteronyssinus* by chromatofocusing. *Int. Archs Allergy appl. Immun.* 69: 224-230 (1982).
- 7 Simpson, R.J.; Moritz, R.L.; Nice, E.C.; Grego, B.; Yoshizaki, F.; Sugimura, Y.; Freeman, H.C.; Murata, M.: Complete amino acid sequence of plastocyanin from a green alga, *Enteromorpha prolifera*. *Eur. J. Biochem.* 157: 497-506 (1986).
- 8 Stewart, G.A.; Johnson, P.M.; Barrett, M.W.; Scopes, P.M.; Stanworth, D.R.: Circular dichroism studies on proteolytic cleavage fragments from rabbit IgG. *Immunochemistry* 14: 263-268 (1977).
- 9 Greenfield, N.; Fasman, G.D.: Computed circular dichroism spectra for the evaluation of protein conformation. *Biochemistry* 10: 4108-4115 (1969).
- 10 Chapman, M.D.; Platts-Mills, T.A.E.: Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus* antigen P₁. *J. Immun.* 125: 587-592 (1980).
- 11 Chapman, M.D.; Tovey, E.R.; Wilkins, S.R.; Platts-Mills, T.A.E.: The allergens responsible for house dust mite allergy. 11th Int. Congr. Allergology and Clinical Immunology, pp. 531-535 (MacMillan, London 1983).

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