

A comparative study of three serine proteases from *Dermatophagoides pteronyssinus* and *D. farinae*

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Studies have shown that the dust mites *Dermatophagoides pteronyssinus* and *D. farinae* contain several serine proteases, two of which have been shown to be allergenic, and to include trypsin and chymotrypsin, corresponding to the groups III and VI mite allergens. However, mites also contain other serine proteases, and the data reported in this study show that an elastase-like enzyme is present in both species. This enzyme was differentiated from the other serine proteases, particularly chymotrypsin, on the basis of charge, substrate specificity, and inhibition by copper and mercury cations. Its apparent mol. mass, as judged by gel filtration, was similar to those previously described for trypsin and chymotrypsin, i.e., 30 kDa. Several isoforms were detected by isoelectric focusing, but the isoelectric points of the major forms in both *D. pteronyssinus* and *D. farinae* were 10.5 and 9.8, respectively, contrasting with the acidic mite chymotrypsins. All three serine proteases were detected in whole mite and faecally enriched extracts, but the activities of trypsin and the elastase-like enzyme were greater in the latter type of extract. These data were similar to those obtained by quantitative immunochemical analysis of the *D. farinae* group III allergen in appropriate extracts, suggesting that culture conditions may modulate protease production. A monoclonal antibody affinity matrix specific for the group III allergen from *D. farinae* was shown to bind mite trypsin. However, a small amount of mite chymotrypsin also bound, suggesting limited immunologic cross-reactivity, a finding consistent with known sequence data.

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Studies from this laboratory and elsewhere have shown that many of the allergens from the clinically important dust mites are enzymatically active. They are found in both whole mites and in faecal particles, suggesting that they are associated with mite digestive processes (25, 29, 31). So far, the allergenic enzymes have all been shown to be hydrolases, and various proteases and carbohydrases have been detected. They include the group I cysteine proteases, the group III trypsins, the group IV amylases, the group VI chymotrypsins, and the group II allergens, which show properties suggesting they are lysozymes (10, 19, 24, 27, 32). However, the relative importance of each of these allergens in the causation of allergic disease remains to be determined.

Of the above allergenic enzyme groupings, relatively little is known about the serine proteases be-

cause most studies performed so far have focused on the clinically significant major groups I and II allergens (21). However, trypsin and chymotrypsin have been detected in both pyroglyphid and nonpyroglyphid mite species (5, 6), and it has been established that each enzyme is allergenic. In this regard, the frequency with which they are recognized in different mite-allergic populations, particularly the group III trypsin allergens, varies from 16 to 100% (3, 16, 27). As yet, it is unclear whether this variation reflects differences associated with the allergic population studied, or the method used to assess allergenicity. In addition, there are data suggesting that the group III allergens may be heterogeneous, particularly on the basis of immunoblotting studies (3), in which a doublet is frequently observed.

Previous studies from our laboratory have shown

that mite extracts contain a serine protease which appears to be unrelated to either trypsin or chymotrypsin on the basis of substrate specificity, charge characteristics, and mol. mass (25). It was found in both whole-mite and faecal-pellet-enriched extracts of dust mites, but little is known of its biochemical and allergenic properties. On the basis of these data, we have set out to characterize this protease, and a description of its biochemical properties form the basis of this paper. These studies confirmed that the enzyme is distinct from the previously described serine proteases, but is similar to a recently reported insect protease showing elastase-like substrate specificity (8, 9). In addition, we have sought to confirm previous preliminary observations that the concentrations of serine proteases are lower in whole-mite extracts than in corresponding faecal extracts, in contrast to observations with mite carbohydrase enzymes such as amylase.

Material and methods

Mite extracts and chemicals

Extracts of *Dermatophagoides pteronyssinus* and *D. farinae* were prepared from both whole mites and spent growth medium (SGM) devoid of mites. Two extracts of *D. pteronyssinus* mites were prepared, one using mites previously washed with organic solvent, and the other using unwashed mites contaminated with growth medium. The *D. farinae* whole-mite extract was prepared from organic solvent prewashed mites. An extract of unused medium (UGM) used in the preparation of both species was also examined. All extracts were prepared as described previously (25). General chemicals and synthetic protease substrates were purchased from Sigma Chemical Co. (St Louis, MO, USA) except where otherwise stated.

Enzyme analyses

Nonspecific substrates. Serine and cysteine protease activities were determined with either insoluble diazotized collagen substrate (Azocoll, Calbiochem, La Jolla, CA, USA (7) or azocasein (30). The results were expressed in units (U) in which 1 U represented the optical density (OD) $\times$ 1000/incubation time in min $\times$ protein concentration in mg. In addition to these assays, the ability of mite proteases to induce casein clotting was also determined, as previously described (15).

Trypsin, chymotrypsin, and elastase-like enzymes. Specific tryptic activity was determined with *N*-benzoyl-arginine-*p*-nitroanilide (BAPNA), whereas specific chymotryptic activity was determined with *N*-benzoyl-tyrosine-*p*-nitroanilide (BTpNA) or succinyl-alanyl-alanyl-prolyl-phenylalanyl-*p*-nitroanil-

ide (SA₂PFpNA) (12, 14). In addition, the presence of proteases capable of hydrolysing the elastase substrates, elastin-orcein, succinyl-alanyl-alanyl-alanyl-*p*-nitroanilide (SA₃-pNA), and succinyl-alanyl-alanyl-prolyl-leucyl-*p*-nitroanilide (SA₂PLpNA) was also assessed, as previously described (4, 8, 11). The data were expressed as the amount of substrate hydrolysed in nmoles per min/mg protein. Protease inhibition experiments were performed with *D. pteronyssinus* SGM at concentrations ranging from 5 to 20 mg/ml. Samples were pretreated with inhibitor for 30–60 min at room temperature before addition of substrate. The inhibitors soybean trypsin inhibitor (SBTI), EDTA, 1,10-phenanthroline, dithiothreitol (DTT) (Boehringer Mannheim, Sydney, Australia), glutathione, aprotonin (Bayer, Leverkusen, Germany), tosyl lysyl chloromethyl ketone (TLCK), tosyl phenylalanyl chloromethyl ketone (TPCK), phenylmethylsulphonyl fluoride (PMSF), benzamidine, a range of divalent cations, human α_1 -antiprotease, and bovine α_2 -macroglobulin (Boehringer Mannheim, Sydney, Australia) were assessed.

Physicochemical characterization of enzymes

Determination of isoelectric point. The isoelectric points (pI) of individual enzymes were determined either by chromatofocusing at 4°C, as previously described (25, 27), or by preparative isoelectric focusing using the Rotofor apparatus (Bio-Rad, North Ryde, NSW, Australia), according to the manufacturer's instructions. Appropriate fractions were collected and analysed for enzyme activity, as described above.

Protein determination. The protein concentrations of individual samples were determined by the Lowry modification of the Folin assay, as previously described (20), with bovine serum albumin as the standard.

Gel filtration studies. Extracts were fractionated on Sephadex G-75, and the apparent mol. mass of the enzymes was determined, as previously described (2), with the mol. mass standards; ovalbumin (43 kDa), chymotrypsinogen (25.5 kDa), and cytochrome C (11.7 kDa).

Effect of acid and heat treatment on mite proteases. Samples of *D. pteronyssinus* SGM were dissolved in a variety of buffers and incubated for 1 h at room temperature. They were then neutralized, centrifuged, and assayed for trypsin, elastase-like enzyme, and chymotrypsin activities, as described above. The effect of heating at 65°C for varying incubation periods was also investigated. Lysozyme activity was assayed as a control because previous studies have

shown that this enzyme is resistant to heat and acid treatment (24).

Soybean trypsin inhibitor (SBTI) affinity chromatography. The serine proteases were isolated by SBTI-Sepharose 4B affinity chromatography. Sepharose 4B was activated with CNBr and coupled with SBTI at a concentration of 5 mg/ml of packed gel at 4°C overnight. The matrix was equilibrated in 0.1 M Tris buffer, pH 8, and affinity chromatography was performed at 4°C. The bound material was eluted with either water adjusted to pH 11 with ammonium hydroxide or 0.1 M glycine adjusted to pH 2.8 with HCl. The resulting fractions were immediately neutralized and assayed for enzyme activity.

Monoclonal anti-Der f III affinity chromatography. *Der f* III was isolated by monoclonal antibody-Sepharose 4B affinity chromatography, as previously described (16). In brief, the column was equilibrated with 0.01 M phosphate buffer, pH 7.2, containing 0.5 M NaCl and 0.1% (v/v) Tween 20, and SGM in the same buffer was applied to the column. The unbound material was eluted, and the column was then washed with 0.01 M phosphate buffer, pH 7.2, containing 0.15 M NaCl. The bound material was eluted with water adjusted to pH 11 with ammonium hydroxide. The proteolytic activities of the eluted fractions were then determined.

Immunochemical quantitation of the group III allergen. The concentration of the trypsin-like group III allergen in *D. farinae* extracts was determined by an inhibition enzyme-linked immunosorbent assay (ELISA). In brief, microtitre plates (EIA plates, Flow Laboratories, McLean, VA, USA) were coated with *Der f* III (2 µg/ml) dissolved in carbonate buffer, pH 9.6. After overnight coating at 4°C, plates were

washed and used immediately in the inhibition experiment. To aliquots of a rabbit anti-*Der f* III antiserum (diluted 1:365 in 0.01 M phosphate buffer, pH 7.2, containing 0.15 M NaCl and 0.1% v/v Tween 20) were added varying concentrations of inhibitor (*Der f* III, *D. farinae* SGM, and *D. farinae* whole-mite extract), and the mixtures were incubated at 4°C overnight. Aliquots were then added to the microtitre plates and incubated at room temperature for 2 h. Plates were then washed and incubated with horseradish peroxidase conjugated antirabbit IgG (Silenus Laboratories, Melbourne, Australia) for 2 h. After washing, the plates were incubated with 3,3',5,5'-tetramethylbenzidine substrate (Kirkegaard and Perry Labs, Gaithersburg, MD, USA), and the concentration of *Der f* III in each of the extracts was determined after reading the OD at 630 nm.

Results

Detection of proteolytic activity

Serine protease activity was detected in all mite extracts with both nonspecific and specific substrates (Table 1). Trypsin and chymotryptic activities, as well as an elastase-like activity, were also detected. The greatest concentrations of each of the three specific serine proteases were found in extracts prepared from SGM rather than whole mite extracts (with the exception of the unwashed whole-mite extract, which was contaminated with SGM). The relative increase in general protease activity in SGM from *D. pteronyssinus* and *D. farinae*, as compared with similar extracts prepared from whole mites, ranged from 2.9- to 7.2-fold. The relative increase in the three individual enzymes ranged from 4.3- to 151-fold. The elastase-like activity was most easily detected with the SA₂PLpNA substrate rather than

Table 1. Quantitative determination of proteases present in dust-mite extracts

Type of extract	Protease activity with substrates						
	General protease		Trypsin BApNA	Chymotrypsin SA ₂ PFpNA	Elastase		
	Azocoll	Azocasein			SA ₃ pNA	SA ₂ PLpNA	Elastin-orcin
<i>D. pteronyssinus</i>							
¹ Whole mite	16.8	43.6	1.2	0.0	1.3	0.8	0.1
² Whole mite	5.5	19.9	0.2	0.0	0.0	0.0	0.0
Spent growth medium	24.6	116.0	3.4	1.5	0.2	22.1	0.5
<i>D. farinae</i>							
² Whole mite	5.4	12.6	0.6	0.0	0.4	0.6	0.1
Spent growth medium	15.7	91.8	2.6	0.9	0.2	13.5	0.2
Unused growth medium	2.2	0.0	0.0	0.0	0.3	0.8	0.0

¹ Whole-mite extract prepared without prewashing of mites in organic solvent.

² Whole-mite extract prepared from mites washed with organic solvent.

Activity obtained with synthetic substrates expressed in nmoles/mg protein, activities obtained with azocasein and azocoll, and elastin-orcin expressed in units/mg protein.

the conventional elastase substrates, SA₃pNA and elastin-orcein (Table 1).

Characterization of trypsin and chymotrypsin

Studies showed that the G-75 gel filtration elution profiles for trypsin and chymotrypsin were similar for both *D. pteronyssinus* and *D. farinae* (data not shown). Two peaks of activity were observed for each enzyme: a minor peak close to the void volume and a major peak with an apparent mol. mass of 30 kDa. Chromatofocusing and isoelectric focusing studies showed that, in accordance with previous studies from our laboratory (27), the trypsin from *D. pteronyssinus* was the most heterogeneous, with major isoforms with pI ranging from 4.8 to 8.6 (Fig. 1 and Table 2). In contrast, trypsin from *D. farinae*, and chymotrypsins from both mite species were less heterogeneous, and all isoforms were acidic (Fig. 1 and Table 2).

The ability of various protease inhibitors to inhibit the activities of trypsin and chymotrypsin was investigated (Table 3). Trypsin activity was completely

Table 2. Isoelectric points of major mite serine proteases from *D. pteronyssinus* and *D. farinae*

Serine protease	Mite species	
	<i>D. pteronyssinus</i>	<i>D. farinae</i>
Trypsin	4.8, 6.2, 8.6	4.8
Chymotrypsin	3.8, 4.4	3.7–4.1
Elastase-like	4.8, 7.8, 10.5	9.8, 4.4

pI data based on both chromatofocusing and isoelectric focusing experiments.

inhibited by α_1 -antiprotease, aprotonin, SBTI, benzamidine, TLCK, and copper and mercury cations, and partially inhibited by PMSF and EDTA. Chymotrypsin was inhibited by α_1 -antiprotease, aprotonin, SBTI, PMSF, EDTA, and copper and mercury cations.

Characterization of elastase-like enzyme

Chromatofocusing and isoelectric focusing studies revealed the presence of an acidic and alkaline enzyme in SGM from both *D. pteronyssinus* and

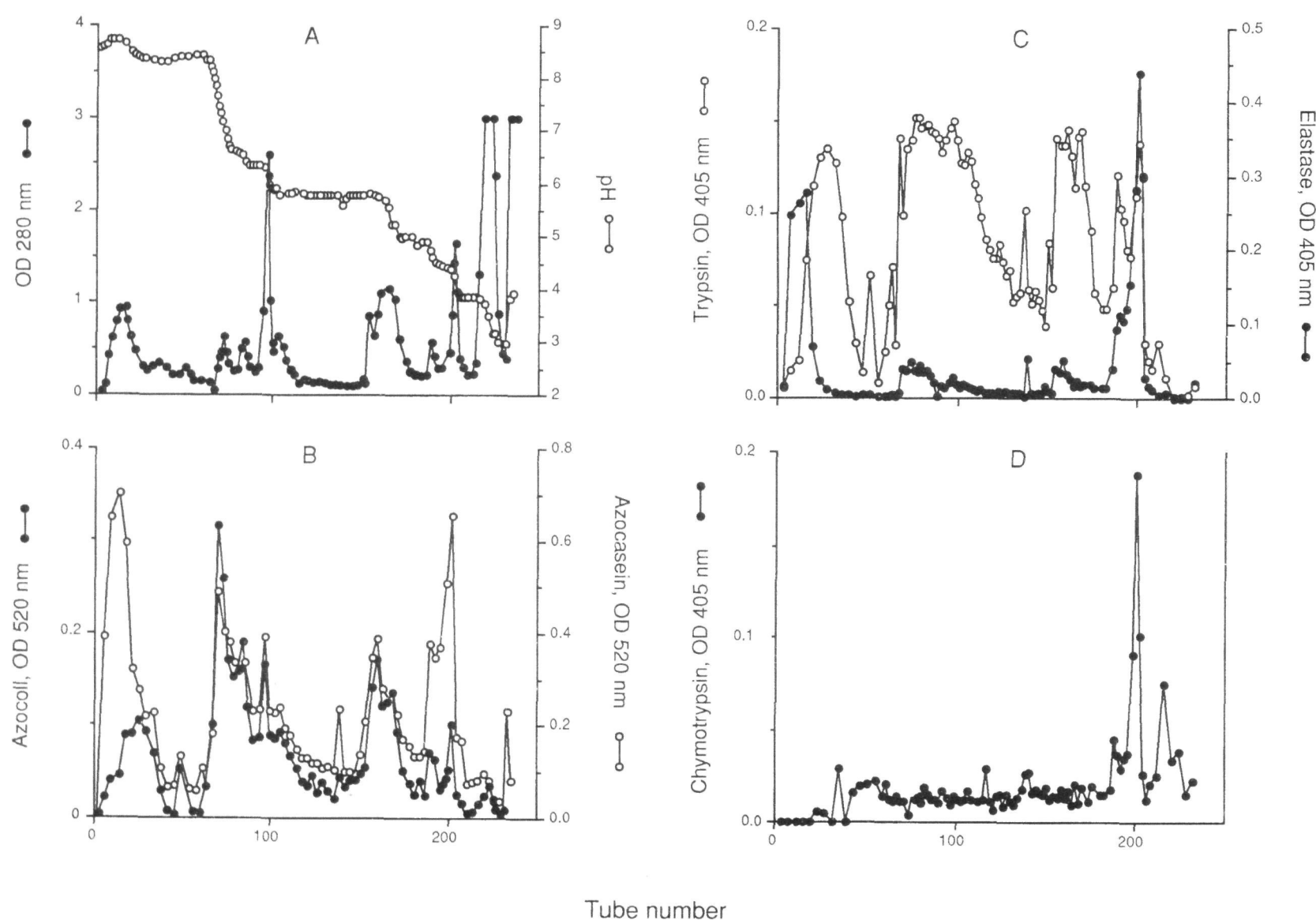


Fig. 1. Chromatofocusing analyses of serine proteases from *D. pteronyssinus*. Eluted fractions were monitored for protein and pH (panel A), general protease activity with azocoll and azocasein (panel B), trypsin and elastase activity with BApNA and SA₂PLpNA, respectively (panel C), and chymotrypsin activity with BTpNA (panel D).

Table 3. Effect of various protease inhibitors on *D. pteronyssinus* proteases

Inhibitor	Percent inhibition of enzyme activity			
	General protease	Trypsin	Elastase-like	Chymotrypsin
α_2 -Macroglobulin (5 μ M)	100	—	—	—
α_1 -Antiprotease (3.9 μ M)	100	100	100	100
Aprotinin (0.2 μ M)	100	100	100	100
SBTI (1.7 μ M)	100	100	100	100
PMSF (8 mM)	78	50	100	100
TLCK (1 mM)	—	100	0	0
TPCK (3 mM)	—	0	0	0
Benzamidine (7 mM)	—	100	0	0
Glutathione (7.5 mM)	—	18	0	0
DTT (7.5 mM)	—	—22	15	15
EDTA (50 mM)	—	49	32	100
1,10-Phenanthroline (50 mM)	—	0	35	30
Cations				
Cu (50 mM)	—	100	90	100
Hg (50 mM)	—	100	100	60

Figures in parentheses are either minimum concentration of inhibitor required to induce maximum inhibition with at least one protease, or maximum concentration of inhibitor used in assay. They refer to concentrations in spent growth medium extract sample before addition of substrate. Trypsin, elastase-like, and chymotrypsin-like activities were determined with BApNA, SA₂PLpNA, and SA₂PFpNA, respectively, whereas general protease activity was determined with azocoll. Dash indicates that inhibitor was not tested.

D. farinae with a specificity for the elastase substrate, SA₂PLpNA (Fig. 1 and Table 2). A similar distribution was also obtained with the chymotrypsin substrate SA₂PFpNA (data not shown), but not with the chymotrypsin substrate BTpNA (Fig. 1), indicating that the elastase-like enzyme corresponded to the alkaline protein. In support of this, chromatofocusing fractions containing this activity, but not those containing the acidic chymotrypsin activity, induced clotting of casein and showed limited activity with elastin-orcein (data not shown). The elastase-like enzyme was shown to have a mol. mass distribution like those of trypsin and chymotrypsin on gel filtration.

Both the acid chymotrypsin and the alkaline elastase-like enzyme corresponded to proteases showing greater activity toward azocasein than to azocoll (Fig. 1), but neither showed activity toward the conventional elastase substrate, SA₃pNA. The elastase-like enzyme in *D. pteronyssinus* SGM was inhibited by an array of inhibitors like that observed with chymotrypsin, with the exceptions, however, of the cations and the chelating agent EDTA, with which clear differences were apparent (Table 3). The elastase-like enzyme also bound to the SBTI affinity matrix (data not shown).

Effect of heat and acid treatment on mite serine proteases

Studies showed that all three serine proteases in SGM were susceptible to acid treatment. Little or no

activity remained after treating samples for 1 h with 0.1 M glycine-HCl at pH 2.8, and a 30–40% fall in activity was shown after treatment with 0.1 M acetate buffer, pH 4.5. All protease activity was lost after treating SGM at 65°C for 200 s. In contrast, the enzymatic activity of mite lysozyme was unaffected by either treatment.

Binding of mite proteases to anti-Der f III affinity column

Many serine proteases show marked sequence similarities, and, on this basis, experiments were performed to determine whether either the chymotrypsin or the elastase-like enzyme present in *D. farinae* SGM is recognized by an anti-*D. farinae* trypsin monoclonal antibody affinity matrix. These studies showed (Fig. 2) that trypsin is the major protease bound (76% of total activity applied). However, a small amount of chymotrypsin also bound (24% of that applied), and there was evidence of trailing, suggesting low-affinity binding of this enzyme to the column. In contrast, binding of the elastase-like enzyme to the column was not detected.

Der f III concentration in whole-mite and spent growth medium

The concentrations of Der f III in the two types of available mite extract were determined by inhibition ELISA. These studies showed that the concentration of this allergen was 149-fold more concentrated in the SGM extract (31.4 μ g/mg) than in the whole-mite extract (0.2 μ g/mg). Enzymatic analyses revealed a 25-fold increase in enzymatic activity in SGM, as compared with whole-mite extract. SGM

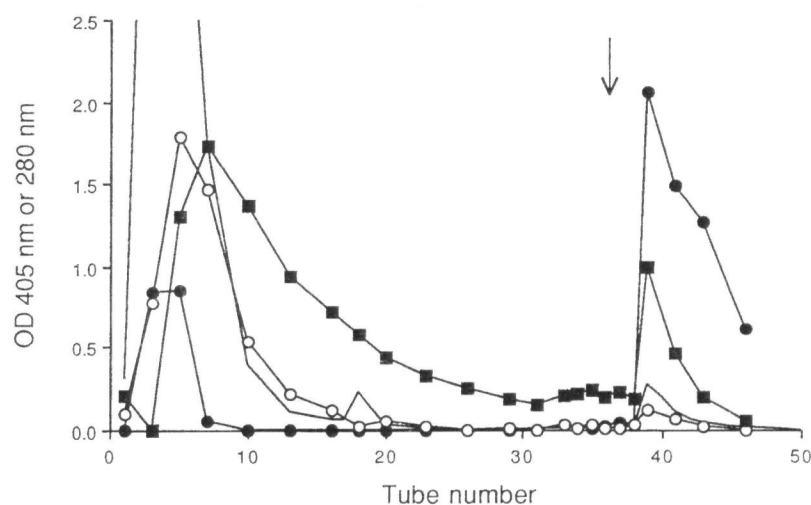


Fig. 2. Binding of serine proteases from *D. farinae* SGM extract (590 mg) to monoclonal anti-Der f III-Sepharose affinity chromatography column. Fractions were examined for protein at 280 nm (—), for trypsin activity with BApNA (●), for chymotrypsin activity with SA₂PFpNA (■), and for elastase activity with SA₂PLpNA (○) at 405 nm. Arrow indicates application of water at pH 11.

or whole-mite extracts of *D. pteronyssinus* did not inhibit this assay although they contained trypsin activity (data not shown).

Discussion

The serine proteases present in extracts prepared from mites belonging to the genus *Dermatophagoides* have been studied by a number of research groups to determine their diversity and specificity in parallel to assessing their allergenicity (17, 23, 25–28). Thus far, two serine proteases (groups III and VI allergens) have been described (27, 28, 32), but, unlike the group I cysteine proteases, our knowledge of these enzymes is relatively limited. However, accumulated data indicate that the serine proteases correspond to trypsin and chymotrypsin on the basis of sequence homology, substrate specificity, and inhibition profiles.

In addition to the above enzymes, data from this study clearly indicate that pyroglyphid dust-mite extracts contain a third serine protease. The enzyme was distinguished from the other two serine proteases principally by its reactivity with the substrates SA₂PLpNA and casein, together with its isoelectric point, and its partial and complete inhibition by EDTA and mercury, respectively. The failure of the enzyme to cleave BTpNA, despite cleaving SA₂PFpNA, also suggested that the elastase-like enzyme was distinct from chymotrypsin, which often shows a similar cleavage spectrum [11]. This protease was similar to the elastase-like enzyme previously shown to be present in orthopteran, coleopteran, and lepidopteran insect species on the basis of both substrate specificity (casein and SA₂PLpNA) and charge (8, 9). As yet, it is not clear whether either the mite or insect enzymes are analogous to other elastases, but its lack of significant reactivity to SA₃pNA and elastin-orcin suggests that it is different; a definitive answer must await sequence homology studies.

Each of the three enzymes described in this report was shown to be a serine protease by its reactivity with inhibitors such as PMSF, aprotonin, α_1 -antiprotease, and SBTI. They were all sensitive to heat and acid treatment, but it is not clear whether the loss of activity was accompanied by marked structural alterations sufficient to affect allergenicity. Calcium ions appeared to be involved in the stabilization of enzymatic activity because EDTA, but not 1,10-phenanthroline (which has a higher affinity for zinc (22)), was shown either to reduce (trypsin and elastase-like enzymes) or completely inhibit (chymotrypsin) enzyme activity.

The apparent mol. mass of trypsin and chymotrypsin, but not the elastase-like enzyme, have previously been reported and shown to be 30 and

25 kDa, respectively (27, 32). On the basis of the gel-filtration data reported here, the elastase-like enzyme is likely to correspond to the 30-kDa protein identified in studies performed with 1,3-[³H] diisopropyl phosphofluoridate (25). The immunochemical relationship between each of the three serine proteases is yet to be determined, but the monoclonal anti-*Der f* III antibody was shown to react in a limited way to mite chymotrypsin. These data suggest that the amino acid sequences of both enzymes are similar, and this conclusion is supported by limited *N*-terminal amino acid sequence data (Table 4). However, the possibility that the chymotrypsin bound by virtue of its proteolytic activity cannot be discounted, although this is considered to be unlikely.

Data from the studies described here showed that each of the three proteases was present in extracts prepared from both whole mite and faeces, but enzymatic activities appeared to be markedly lower in body extracts. These findings agree with those reported earlier for trypsin, but not for chymotrypsin, which was found to be present in similar concentrations in whole-mite and faecal extracts when esterase activity was used as a marker (23). Immunochemical data obtained with a specific anti-*Der f* III antiserum support the biochemical findings obtained for trypsin, but the cause(s) of the discrepancy in the protease content of the two types of extract is unclear.

It is unlikely that denaturation of mite proteases by organic solvents used in the harvesting of whole mites (but not SGM) accounted for the differences detected, because similar effects were not observed with other mite enzymes, including *Der p* I, amylase (*Der p* IV), glucoamylase, or lysozyme (23). The most likely explanation is that the synthesis of certain proteases is specifically modulated. In support of this, it has been shown that insect protease production is particularly susceptible to induction and repression in response to diet (13). It is feasible, therefore, that as the mite growth medium becomes spent because of mite population expansion, the production of certain enzymes is repressed. Irrespective of the reasons, however, such findings may have some relevance for immunochemical analyses of mite extracts, the production of representative skin test reagents, and the importance of specific allergens to the allergic population.

Both mite trypsin and chymotrypsin have been shown to be allergenic although there is some confusion regarding the frequency of reactivity (3, 16, 27). The allergenicity of the elastase-like enzyme has not yet been reported, but preliminary RAST data (King, Thompson, Stewart, unpublished observations) indicate that this enzyme is allergenic also, with a frequency of reactivity similar to that of

Table 4. N-terminal sequences of mite serine proteases of *D. pteronyssinus* and *D. farinae*

Protease	Sequence																			
Mite trypsin	1																			20
<i>Der f</i> III	I	V	G	G	V	K	A	Q	A	G	D	X	P	Y	Q	I	S	L	Q	S
<i>Der f</i> III	I	V	G	G	V	K	A	L	A	G	D	X	P	Y	E	I	S	L	E	V
Df protease	I	V	G	G	V	K	A	Q	A	G	D	X	P	Y	Q	I	S	L	Q	S
Df trypsin	I	V	G	G	V	K	A	Q	A	G	D	X	P	Y	Q	I	S	L	Q	S
<i>Der p</i> III	I	V	G	G	E	K	A	L	A	G	E	X	P	Y	Q	I	S	L		
Mite chymotrypsin																				
Df chymotrypsin	(A)	(V)	G	G	Q	D	A	D	L	A	E	A	P	F	Q	I	S	L	L	K
Dp chymotrypsin	(A)	I	G	X	Q	(P)	A	A	E	(A)	(E)	A	P	F	Q	I	(S)	L	M	K

One letter code used throughout. X indicates that residue could not be determined. Letters in parentheses indicate uncertainty. Residues showing identity with any trypsin-like mite allergen are indicated by bold type, whereas residues considered to be chemically similar (A, S, T; D, E; N, Q; I, L, M, V; F, Y, W) are underlined. Sequences were obtained from References 1, 16, 18, 27, 32.

trypsin. If confirmed, the data may account for the observations obtained from immunoblotting studies showing that two proteins with mol. mass similar to those of trypsin and the elastase-like enzyme bound IgE from most allergic donors (3). However, more studies are required with purified allergens and a panel of sera.

In conclusion, the data presented in this report indicate that pyroglyphid dust-mite extracts contain at least three serine proteases with physicochemical and enzymatic properties similar to those described for enzymes derived from vertebrate and nonvertebrate sources. The data indicate that chymotrypsin, trypsin, and the elastase-like enzyme are present at relatively high concentration in mite faecal particles, and all must, therefore, be regarded as being potentially allergenic, although their relative importance is yet to be determined. At present, it is not clear whether the elastase-like protease is a feature of all mite species, but preliminary data indicate that non-pyroglyphid mites such as *Lepidoglyphus destructor* (Stewart, van Hage-Hamsten, Olsson, Thompson, unpublished observations) also contain the enzyme, suggesting that this and the other serine proteases are widespread.

Acknowledgments

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