



Detection of serine proteases in extracts of the domestic mite *Blomia tropicalis*

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Abstract. Previous studies have shown that the domestic mites *Dermatophagoides pteronyssinus* and *D. farinae* contain allergens with serine protease activity. These proteolytic allergens include trypsin, chymotrypsin, elastase, kallikrein, and C3/C5 convertase. However, it is not known whether the domestic mite *Blomia tropicalis* shares with other mite species the serine protease activities. The enzymatic activity present in extracts obtained from food-free *B. tropicalis* was investigated using specific substrates and inhibitors. Based upon the concentration response and inhibition profiles, and the digestion of specific substrates our data demonstrate that extracts from *B. tropicalis* exhibit several serine-protease-like activities. The enzyme activities detected in the *B. tropicalis* extracts are trypsin, elastase, chymotrypsin, kallikrein, C3/C5 convertase, and mast cell protease. Our results also demonstrate that kallikrein and C3/C5 convertase-like activities were not significantly affected by the α_1 -antiprotease, a naturally occurring serine protease inhibitor which protects lung mucosa from the enzymatic action. These data strongly suggest that the Echymyopodidae mite *B. tropicalis* shares at least five serine proteases with members of other mite families, the Glycyphagidae and Pyroglyphidae. In addition, our data demonstrate the potential use of biochemical methods to detect serine proteases for evaluation of mite growth *in vitro*, or to detect environmental exposures to these enzymes.

Introduction

The subclass Acari, which includes ticks and mites, contains over 35,000 described species (Colloff 1993). Of these, 300 species are associated with humans as pests of stored products, commensals or parasites. Several mite species live in household dust and are termed “domestic mites”. Some of their products are an important source of allergens for humans. Domestic mites have developed a diversity of enzymes needed to digest the different types of food found in their habitats. These enzymes include carbohydrases, proteases, phosphatases and esterases/lipases. Some of these enzymes are important in human health because they have the capacity to participate in both allergic and non-allergic mechanisms of pathogenesis in atopic disease (Robinson et al. 1997).

Serine proteases is one of the most studied group of proteolytic enzymes, and are found in vertebrates as well as in molds, ragweed pollen, cockroaches, domes-

tic mites and bacteria. (Yu et al. 1999; Bagarozzi and Travis 1998; Wongtim et al. 1993; Stewart et al. 1994). Serine proteases can act as exopeptidases, endopeptidases, oligopeptidases, and omega peptidases. Thus, they may have their function in the vicinity of the cells producing them digesting matrix proteins, or they may be released into the body fluids to exert their action as zymogen activators far from the secreting cells (Rawlings and Barret 1984). In addition to their function in digesting matrix proteins and as zymogen activators, some serine proteases (trypsin, chymotrypsin and elastase) play an important role as digestive enzymes in vertebrates and invertebrates, including mites.

Initial descriptions suggesting the biological importance in humans of the mite proteolytic allergens were conducted by Stewart et al. (1989), who showed that a purified allergen In addition to their function in digesting matrix proteins and as zymogen activators, some serine proteases (trypsin, chymotrypsin and elastase) play an important role as digestive enzymes in vertebrates and invertebrates, including mites. from the domestic mite *D. pteronyssinus* (Dp) had homology with and proteolytic activity compatible with the serine proteases trypsin and chymotrypsin. In other *in vitro* studies, Herbert et al. (1989) demonstrated that exposure of bronchial mucosa to a purified serine proteolytic allergen causes an increase in its permeability. More recently, Tomee et al. (1998) induced cell detachment and an increase in the production of proinflammatory cytokines in respiratory epithelial cells treated with serine proteases obtained from the domestic mites Dp and *Lepidoglyphus destructor* (Ld). Serine proteases also have been described in Dp (Nishiyama et al. 1996) and presumably have the same biological functions as those found in Dp.

Several mite species contribute to allergen mixtures in household dust, and *Blomia tropicalis* (Bt) is an important source of allergens in tropical and subtropical regions and in the south coastal regions of the United States. (García-Ibañez et al. 1991; Vargas and Mairena 1991; Hurtado and Parini 1987; Arlian et al. 1992) Evidence supporting the allergenicity and clinical importance of Bt in atopic diseases especially in asthma and allergic rhinitis, has been provided by several authors. (Arruda et al. 1991; Gabriel et al. 1982; Montealegre et al. 1998). Although there are available data on its world distribution and role as sensitizing agent in atopic disease, little is known of its proteolytic allergen diversity. Preliminary findings have suggested that Bt has a rich and diverse enzyme content, similar to other domestic mite species. (Montealegre et al. 1997). The objective of the present work was to detect the presence of serine proteases with biological relevance in allergy, in extracts obtained from food-free Bt bodies.

Materials and methods

Purification of mite bodies

The domestic mite Bt was grown in 8 inch diameter culture dishes on TetraFin fish food (TetraWerke, Blacksburg, VA). After 28 days, live cultures containing mites,

fecal particles and culture media were placed on a funnel with 150 size mesh and exposed to a 40 watt light bulb for 4 h. The live mites, which dropped through the mesh, were collected in 50 ml conical centrifuge tubes, immediately placed on a 1" deep and 12" diameter brass sieve pan (Dual Manufacturing Co, Chicago, IL) and evenly distributed to form a uniform layer and again exposed to the 40 watt light for 15 min. After this period, the brass sieve pan was turned face down and gently tapped so that food particles and dead mites were eliminated. The live mites, which had adhered to the brass sieve pan, were killed by storing the pan at -70°C , and then dried by exposing to a light bulb for 4 h as described above. To detach the food-free dead mites from the pan, an acrylic gel scoop (Sigma, St. Louis, MO) was used to eliminate any remaining food particles, and the dried mites were sieved using three 8" diameter U.S. Standard sieves (Dual Manufacturing Co, Chicago, IL). The mesh openings used were $425\ \mu\text{m}$, $300\ \mu\text{m}$, $250\ \mu\text{m}$ and the dried mites were placed on a sieve shaker (WS. Tyler Inc, Mentor, OH) and agitated for 4 h. The dried mites which stayed between the $300\ \mu\text{m}$ and $250\ \mu\text{m}$ mesh opening and were considered food-free mite bodies. The purity of the preparation was verified by placing 0.5 mg of dried mites on a $4 \times 4\ \text{mm}$ plastic dish and examined under at 20X using a SZ-STB1 stereoscope (Olympus, Japan). The microscopic analysis revealed that the composition of the preparation was 99.5% bodies with fecal particles and 0.5% food particles. Most of the mite bodies had fecal particles within their bodies or attached to their legs.

Extracts

Mite extracts were prepared according to established protocols (Puerta-Lerena et al. 1991; Montealegre et al. 1997). A total of 50 grams of food-free Bt bodies were extracted in one liter of 0.2 M ammonium bicarbonate. The mixture was placed in a beaker and sonicated for 15 min on ice at full output for 10 cycles. After this, the mixture was placed in a refrigerated cabinet and incubated overnight under constant agitation. The extract was centrifuged at 6500 rpm for 1 h, and the resulting supernatant extensively dialyzed against distilled water at 5°C . The extract was concentrated to 50 ml using polyethylene glycol and centrifuged at 15,000 rpm for 1 h. The protein concentration of the clear supernatant was determined using an adaptation of the Lowry assay (Lowry and Rosenbrough 1951). The protein concentration was adjusted to 2 mg/ml, and 1 ml aliquots were stored at -80°C until use. Mite growth media was processed as indicated. As controls for all of the assays, extracts from mite growth media were standardized to $5\ \mu\text{g/ml}$ representing a 0.5% contamination in the mite preparation and tested for enzymatic activity.

Enzyme analyses

Extract concentrations

For the concentration-response studies, mite extracts were serially diluted from 1.0 mg/ml to $0.4\ \mu\text{g/ml}$ in digesting buffer. For the inhibition analyses, the protein concentration was adjusted to 0.35 mg/ml.

Concentration-response using nonspecific substrate

Total proteolytic activity was measured using fluorescent-labeled casein (Molecular Probes, Eugene, OR) according to the manufacturer's instructions. Briefly, Bt extracts were serially diluted from 1.0 mg/ml to 0.4 μ g/ml in 0.01M Tris pH 7.8. The assay was performed in 96 well microtiter plate fluorometer (Dynex Technologies, Chantilly, VA) by mixing 50 μ l of Bt extract with 50 μ l of substrate. The reaction was allowed to proceed for 1 h protected from the light at room temperature. After this period, the reaction was analyzed at 480 nm excitation and 530 emission. Data were expressed as fluorescent units. As a negative control, extracts from culture media free of mites was used, and the obtained values was subtracted from all of the reactions.

End-point concentration-response activity using specific serine protease substrates

Enzyme substrates were purchased from Sigma Chemicals (St. Louis, MO) otherwise indicated. Tryptic activity was determined with N-benzoyl-arginine-p-nitroanilide (BA-pNA), whereas specific chymotryptic activity was determined with N-benzoyl-tyrosine-p-nitroanilide (BT-pNA). The presence of elastase was verified by succinyl-alanyl-alanyl-alanyl-p-nitroanilide (SA₃-pNA) while the kallikrein activity was detected using Z-Phe-Pro-Arg-AFC-2HCl (Enzyme Systems Products, Livermore, CA), mast cell protease with MeO-Suc-Phe-Leu-Phe-AFC (Enzyme System Products, Livermore, CA), and C3/C5 convertase using BOC-Leu-Gly-Arg-AMC-HCl (Calbiochem-Novachem, La Jolla, CA). For the elastase activity, a total of 10 mg of the specific substrate N-succinyl-ala-ala-ala-pro-leu-p-nitroanilide (SA₃-pNA) were dissolved in 2 ml of DMSO. To this preparation, 18 ml elastase assay buffer were added, and 50 μ l of the mite extract were added to the wells of a 96-well micro titer plate, and immediately 200 μ l of the SA₃-pNA substrate solution was dispensed to each well. The plate was immediately at 405 nm wavelength to obtain a first reading OD value at 0 min, and incubated at 37 °C for 60 min to obtain a second reading at 60 min. The reaction was stopped by adding 50 μ l of 50% (v/v) acetic acid to each well. The plate was analyzed using a Microtiter Plate Reader (Benchmark Plate Reader, Bio-Rad Laboratories, Hercules, CA) at 405 nm of wavelength. To measure trypsin, 5 mg of N-benzoyl-DL-arginine p-nitroanilide (BAPNA) was dissolved in 2 ml DMSO under a fume hood and 18 ml trypsin assay buffer was added. A total of 100 μ l of the mite extract and an equal volume of the substrate solution were placed each well. The reaction was analyzed as previously described. For the chymotrypsin assay, 5 mg of N-benzoyl-tyrosine-p-nitroanilide were dissolved in 2 ml of dimethyl sulphoxide (DMSO). To this mixture, 18 ml of digestion assay buffer 0.08M Tris supplemented with 0.1M CaCl₂ at pH 7.8 were added to the substrate. A total of 100 μ l of sample and equal volume of the substrate solution were dispensed to each well. The reaction will be analyzed as described previously described.

For the kallikrein activity, a 20 mM substrate solution was prepared by dissolving 12.14 mg in 1 ml of DMSO. A total of 100 μ l of the stock solution was further diluted in 5 ml of 10 mM Tris-HCl pH 7.4. A total of 50 μ l of mite extract and

equal volume of the substrate solution were dispensed to each well. The reaction was allowed to proceed for 60 min, and was analyzed at 355 nm excitation and 460 nm emission wavelengths. For the mast cell protease, a 20 mM stock solution was made by dissolving 10 mg of the substrate in 600 μ l of DMSO. A total of 20 μ l was diluted in 5 ml of 10 mM Tris-HCl pH 7.4, and the activity was measured as previously described. The C3/C5 convertase was detected by dissolving 12 mg of the substrate in 1 ml of DMSO. A total of 40 μ l of the stock solution was diluted in 5 ml of 10 mM Tris pH 7.4, and the activity was measured as previously described.

End-point inhibition assays

Enzyme inhibitors were purchased from Sigma Chemicals (St. Louis, MO). Protease inhibition experiments were performed with Bt extract at the optimized protein concentration of 0.35 mg/ml. Samples were pre-treated with inhibitor for 60 min at room temperature (20 °C, 40% relative humidity) before addition of the specific substrate. The following protease inhibitors were used: phenyl methyl sulfonyl (PMSF) and α_1 -antiprotease for serine proteases in general, soybean trypsin inhibitor (SBTI), benzamidine and n- α -p-Tosyl-L-Lysine (TLCK) for trypsin, n-tosyl-L-phenylalanine chloromethyl ketone (TPCK) and soy bean-derived chymotrypsin inhibitor for chymotrypsin, ethylenediaminetetraacetic acid for inhibition of metalloproteases (EDTA), dithiothreitol (DTT) for cysteine proteases in general, 1,10 phenanthroline as a general metalloproteinase inhibitor, leupeptidin for both serine and cysteine proteases, and $MgCl_2$, as a chelating agent. In the case of the kallikrein, all of the inhibitors tested were used in the presence of SBTI (Garrett et al. 1985). The assays were conducted as follows. In a low-binding 96-well microtiter plate, samples previously treated with 50 μ l of the inhibitors were incubated with an equal volume of the specific substrate. The enzymatic activity was monitored by an ELISA (Benchmark Plate Reader, Bio-Rad Laboratories, Hercules, CA) plate reader or a fluorescent plate reader (CytoFluor 2350, Millipore Corporation, Malborough, MA). All assays were conducted in quadruplicate. The assays and percent inhibition was calculated as previously described (Stewart et al. 1991).

Data analysis

Data were electronically transferred from the microtiter plate readers to an electronic spreadsheet where it was verified for its integrity or missing values. Then, the data were electronically transferred to Stata (Stata Corporation College Station, Texas) where all of the statistical analyses were performed. For the concentration-response, regression analysis and its diagnostics were conducted (Hamilton 1992). The final model used was $y=a_0+a_1/(1+\exp(-(x-a_2)/a))$.

Results

Concentration-response and proteolytic activity

To determine whether the protein concentration of the mite extract was related to the enzyme activity present in the mite extract, serial dilutions ranging from 0.5 mg/ml to 0.4 μ g/ml were conducted. As shown in Figure 1 (Panels A-G), all of the substrates tested showed a high degree of correlation between enzymatic activity and protein concentration as shown by their adjusted coefficient of determination (R^2). The range of the R^2 in all experiments conducted was from 0.90 to 0.99, with high levels of significance. Of the substrates tested, chymotrypsin, kallikrein and C3/C5 convertase, showed a high degree of proteolytic reactivity. Most of the enzyme activities were detected at extract concentrations of 0.007 mg/ml with the exception of the total proteolytic activity using casein as non-specific substrate.

Effects of inhibitors and metal ions on substrate specificity

Casein digestion

The inhibitory effect of various chemicals on nonspecific protease activity present in extracts from Bt bodies is summarized in Table 1. The protease activity was strongly decreased (>50%) by the typical serine inhibitors PMSF (75.1%) and α_1 -antiprotease (96.7%). Similar results were obtained when trypsin inhibitors TLCK, benzamidine and SBTI, and chymotrypsin inhibitors TPCK and chymotrypsin inhibitor were used. These results indicate that serine protease activity is present in Bt extracts. In contrast, leupeptidin showed low inhibitory capacity reaching 30.7%. As part of the panel of enzyme inhibitors, cysteine proteases, aspartic and metallo proteinases inhibitors were assessed. High inhibitory activity was detected when DTT with general protease substrate was used, while low inhibitory activity was detected with the remaining specific substrates. These results indicate that extracts from food-free bodies from the domestic mite Bt show strong protease activity that it may include serine, cysteine and metallo-like proteases.

Trypsin

Trypsin-like activity was identified by inhibition of the reactivity between Bt extracts and the specific substrate BapNA. The analysis of the data showed that PMSF, α_1 -antiprotease TLCK, benzamidine and soy bean trypsin inhibitor (SBTI) produced high levels of inhibition. Of these, SBTI inhibited 96.2% the enzymatic activity. In addition, TPCK and chymotrypsin inhibitor were also tested, and no inhibitory activity was detected in both cases. Other protease inhibitors tested included leupeptidin. In this case, a high inhibitory activity was observed. No inhibitory activity was detected using cysteine or aspartase inhibitors.

Elastase

Elastase inhibition was evaluated by using the specific substrate SA₃-pNA and the serine inhibitors PMSF and α_1 -antiprotease. In both cases, strong inhibitory activ-

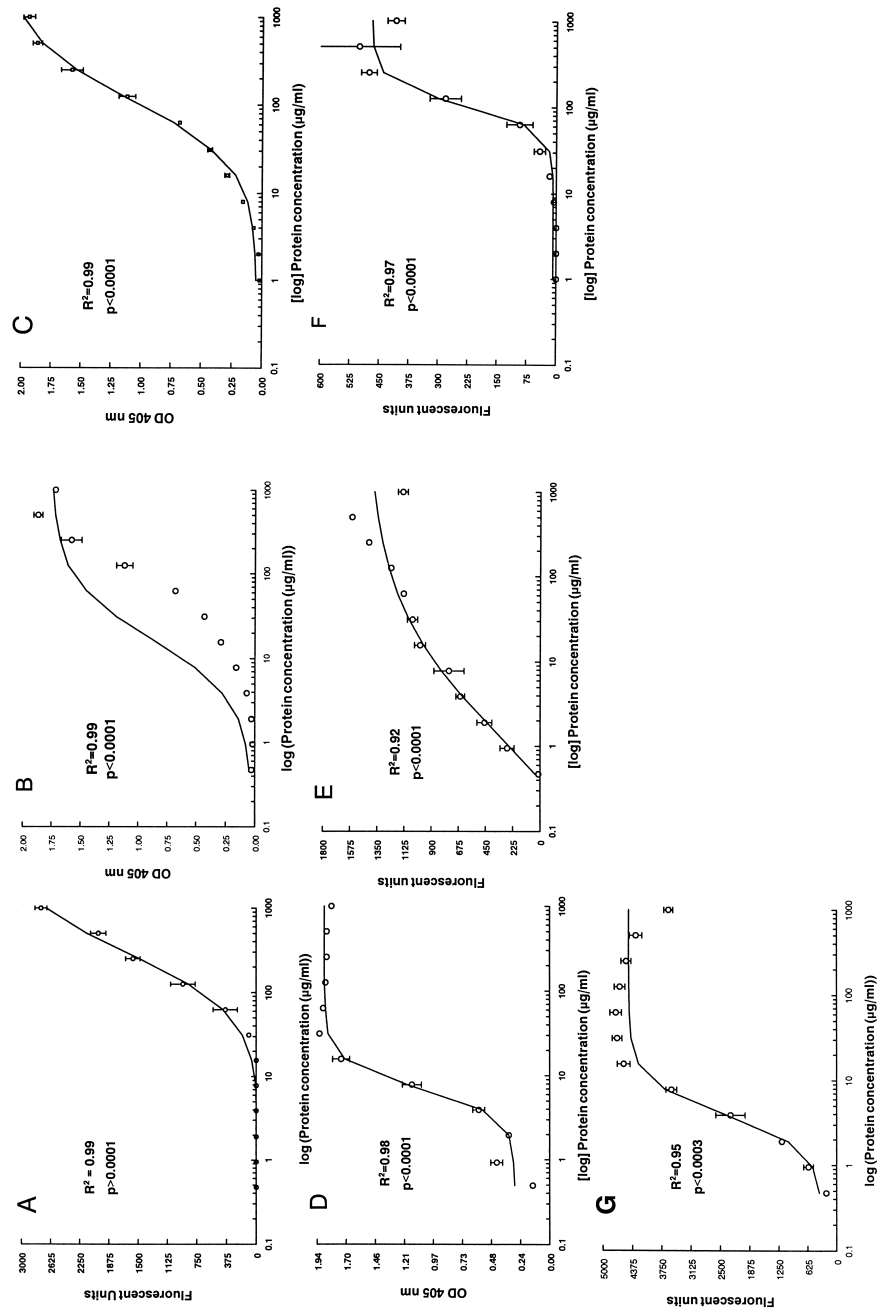


Figure 1. Concentration-response curves of serine protease-like activities and substrates in food-free Bt extracts: A) total proteolytic activity (casein), B) trypsin (BapNA), C) elastase (SA_3 -pNA), D) chymotrypsin (Bt-pNA), E) kallikrein (Z-Phe-Pro-Arg-AFC-2HCl), F) mast cell protease (MeO-Suc-Phe-Leu-Phe-AFC), and G) C3/C5 convertase (BOC-Suc-Phe-Leu-Phe-AFC).

Table 1. Effect of various protease inhibitors on serine protease activities obtained from food-free bodies of *B. tropicalis*.

Inhibitor	Percent inhibition of enzyme activity					
	General protease	Trypsin	Elastase	Chymotrypsin	Kallikrein	Mast cell protease
PMSF (10 mM)**	75.1 ± 5.9*	77.8 ± 1.2	99.4 ± 0.32	99 ± 0.25	65.1 ± 0.5	99.3 ± 0.58
α_1 -antitrypsin (8mg/ml)	96.7 ± 2.3	76.6 ± 4.8	77.9 ± 2.7	99 ± 0.05	17.3 ±	99.0 ± 0.11
SBTI (3 mg/ml)	86.9 ± 6.5	96.2 ± 0.5	0	88.3 ± 0.2	39.3 ± 3.21	98.6 ± 0.2
TLCK (10 mM)	83.1 ± 2.5	85.4 ± 10.0	66.4 ± 2.8	25.2 ± 7.05	67.40 ± 0.2	16.9 ± 4.7
Benzamide (20 mM)	71.7 ± 2.5	72.6 ± 2.8	79.5 ± 2.4	70.2 ± 0.25	0	90.1 ± 4.5
Leupeptidin 10 mM	30.7 ± 7.7	99.3 ± 0.1	82.1 ± 0.81	14.7 ± 5.67	78.0 ± 0.6	4.9 ± 3.4
TPCK (10 mM)	89.2 ± 3.8	0	81.4 ± 4.2	83.7 ± 5.03	0	98.1 ± 0.07
Chymotrypsin inhibitor 1 mg/ml	98.5 ± 0.8	0	2.4 ± 1.7	86.4 ± 1.29	7.5 ± 2.1	0
DTT (10 mM)	79.7 ± 8.7	0	0	26.8 ± 3.49	0	55.4 ± 3.37
EDTA 100 mM	81.6 ± 7.2	28.1 ± 6.1	28.7 ± 6.4	86.8 ± 1.44	0	3.9 ± 1.81
1,10-Phenanthroline 10 mM	70.2 ± 7.3	87.9 ± 7.6	44.5 ± 4.1	15.6 ± 3.83	36.8 ± 1.3	97.1 ± 0.4
MgCl (10 mM)	23.6 ± 3.2	77.7 ± 10.3	0	14.9 ± 6.7	0	74.7 ± 4.57
						6.6 ± 6.0
						0

*Inhibition mean + SD (standard deviation) **Figures in parenthesis indicate maximum concentration of inhibition used in assay.

ity was detected. The enzymatic activity was inhibited by TLCK and benzamidine, but not SBTI. Other protease inhibitors evaluated were TPCK which inhibited the reaction, and the chymotrypsin inhibitor which did not inhibit the reaction. In contrast, leupeptidin was able to inhibit the enzymatic reaction.

Chymotrypsin

The inhibition of the chymotryptic activity was assessed with BT-pNA and known protease inhibitors. The serine inhibitors PMSF and α_1 -antiprotease completely inhibited the chymotrypsin activity. In addition, TLCK produced marginal inhibition, while benzamidine and SBTI showed a strong inhibitory activity. The chymotrypsin inhibitors TPCK (83.7%) and EDTA (86.8%) were effective in inhibiting the chymotrypsin activity. The other protease inhibitors tested produced only a marginal inhibitory action.

Kallikrein

Kallikrein enzymatic activity was inhibited by PMSF. Trypsin inhibitor TLCK (67.4%) induced high inhibition. Leupeptidin, an inhibitor with specificity for both serine and cysteine proteases induced a strong inhibition.

Mast cell protease

Mast cell protease activity was highly inhibited by PMSF (99.3%), α_1 -antiprotease (99.0%), EDTA (97.1%), TPCK (98.1%), SBTI (98.1%), benzamidine (90.1%) and 1,10-phenantroline (74.7%). In contrast, TLCK had minimal inhibitory effects. No inhibitory activity was detected with pepstatin, MgCl, leupeptidin or DTT.

C3/C5 convertase

The enzymatic activity was highly inhibited by the protease inhibitors PMSF, TLCK, TPCK, leupeptidin, moderately inhibited by phenantroline, chymotrypsin inhibitor, and marginally by SBTI, DTT and by α_1 -antiprotease.

Discussion

Data describing serine protease activities with allergenic properties in Echymyopodidae and Glycyphagidae domestic mites are limited. In contrast, in the Pyroglyphidae family members these proteolytic allergens have been studied in some detail (Colloff 1993). In the present report, we have provided evidence based on digestion of specific substrates, concentration-response curves and inhibition profiles that extracts obtained from bodies of the Echymyopodidae mite Bt exhibit several serine protease-like activities including trypsin, chymotrypsin, elastase, kallikrein, mast cell protease, and C3/C5 convertase.

The serine proteases trypsin, chymotrypsin and elastase are found in the digestive tract of the Pyroglyphid mites Dp (Yasueda et al. 1993), Df (Stewart et al. 1989), *Euroglyphus maynei* (Stewart et al. 1992) and the Glycyphagid mites (Ld)

and *Glycyphagus domesticus* (Stewart et al. 1991). As expected, Bt extracts showed enzymatic-like reactivity which could be detected at low protein concentrations, especially chymotrypsin (Figure 1, panels B, C and D). These results, in conjunction with the inhibitory profiles (Table 1) strongly suggest that Bt shares with Dp, *Dermatophagoides farinae* (Df) and *E. maynei*, the known serine proteolytic allergens trypsin, elastase and chymotrypsin (Stewart and Thompson 1996). We also studied other less known serine proteases of mites. These enzymes were kallikrein which cleaves kininogens with resulting peptides involved in the inflammatory cascade (Pesquero and Bader 1998), and C3/C5 convertase which plays an important role in the complement-mediated inflammation. We also included in the present study mast cell proteases because they are potent stimulators of mucous secretions in the human airways (Nadel 1991).

Kallikrein-like activity was detected in the mite extracts using the substrate Z-Phe-Pro-Arg-AFC-2HCl. This substrate has been used to measure kallikrein activity in nasal secretions of allergic patients challenged with domestic-mites (Katori et al. 1996). The concentration-response curve (Figure 1, panel E) shows its enzymatic activity has a direct relationship with the protein concentration of the mite extract. In addition, the inhibition profiles demonstrate that TLCK but not α_1 -antiprotease produced a significant reduction of enzymatic activity. These inhibitory patterns are in agreement with those previously reported in human salivary kallikrein (Fujimoto et al. 1990). The serine protease kallikrein has been described only in Df (Takahashi et al. 1990). The authors demonstrated that a purified polypeptide had kallikrein activity triggering vascular permeability.

The activity of the C3/C5 convertase was detected using the synthetic substrate Boc-Leu-Gly-Arg-AMC, which is highly specific for the C3/C5 convertases (Corporale et al. 1981). Our data showed that concentration-response was also proportional to the protein concentration, and the enzymatic activity was inhibited by leupeptidin (Table 1). This observation is consistent with the inhibitory profiles of C3/C5 convertase previously reported by Corporale et al. (1981). Although the leupeptidin inhibits both serine and cysteine proteases, the use of a highly specific substrate substantiates our results. In domestic mites, this enzyme has been reported in Df by Maruo et al. (1997). The C3/C5 convertase-like activity showed substrate digestion which was detectable at very low protein concentrations (Figure 1, panel G).

Lastly, using the substrate MeO-Suc-Phe-Leu-Phe-AF, we were able to detect mast cell-like protease activity in Bt extracts. The inhibition profile obtained in our experiments shows a significant decrease in the activity induced by the chymotrypsin inhibitor TPCK, SBTI but not with the trypsin inhibitor TLCK. This results are in agreement with those obtained by Hultsch et al. (1998). To our knowledge, the mast cell protease enzymatic activity has not been previously described yet in domestic mites.

The α_1 -antiprotease is a major member of the large family of serum serine protease inhibitors, the serpins, which neutralize serine proteases preventing and controlling adverse inflammatory responses (Carrell 1990; Stone and Le Bonniec 1997; Patston et al. 1994; Patston 2000). An unexpected finding was the fact that the

α_1 -antiprotease failed to neutralize two serine protease activities included in our study. In our experiments we used the α_1 -antiprotease at the concentration of 8 mg/ml, and at this concentration, it was expected that all of the serine protease enzymatic activity would be inhibited (Pannell et al. 1974). Data showed that trypsin, elastase, chymotrypsin and mast cell protease activities were significantly inhibited by human α_1 -antiprotease, but not kallikrein and C3/C5 convertase-like activities (Table 1). The observation that C3/C5-like and kallikrein-like protease activities were not neutralized by the antiprotease could provide an additional explanation as to why mite products are strong inducers of inflammatory or allergic reactions. In fact, it has been reported in an allergic experimental animal model, that there is negative association between α_1 -antiprotease activity and kallikrein activity (Forteza et al. 1996).

Our findings also allow us to classify serine activities into two major groups. The first is composed of those proteolytic allergens that are inhibited by the α_1 -antiprotease, whereas the second is not. In environmental exposures in which serine proteolytic allergens are present in sufficient levels and for long periods of time, those that are not neutralized by the α_1 -antiprotease may penetrate the lung's defenses where they will chronically stimulate cells of the immune system initiating an allergic response or augment the response via the protease receptors, acting as adjuvant molecules of inflammation (Thompson 1998).

The data here presented should provide useful in the biochemical detection and characterization of Bt proteolytic allergens. The use of affinity chromatography for purification of most of the enzymes described in this report should facilitate purification strategies. Also environmental monitoring of serine proteases present in household dust should provide important data on total potency of exposures. To this end, previous work conducted by Berrens (1991) show that there is a quantitative association between proteolytic activity in household dusts and the ability of these extracts to bind IgE antibodies from the serum obtained from patients allergic to domestic mites. In addition, measurement of C3/C5-like and kallikrein-like proteases in environmental studies could prove important markers of risk assessment for asthma or rhinitis.

In conclusion, our data strongly suggest that the domestic mite Bt contains enzymes with at least six serine protease-like activities. In domestic mites, trypsin, elastase and chymotrypsin are enzymes associated with digestion of food, whereas mast cell protease, C3/C5 convertase and kallikrein, their biological function is unknown. All of these enzymes have been previously detected in mites, with the exception of mast cell protease. In this regard, the *Echymyopodidae* mite Bt shares with *Glycyphagidae* and *Pyroglyphidae*, several serine proteases with digestive and proinflammatory properties. Since in our experiments could not provide an absolute measurement of specific enzyme activity, further studies of the serine proteases by immunochemical characterization or cDNA are needed to confirm our observations.

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