



An Enzymatic Analysis of the Storage Mite *Lepidoglyphus destructor*

Geoffrey A. Stewart,¹ Marianne van Hage-Hamsten,² Karen Krska,¹
Philip J. Thompson,³ and Susanna Olsson²

¹DEPARTMENT OF MICROBIOLOGY, THE UNIVERSITY OF WESTERN AUSTRALIA, QE II MEDICAL CENTRE, NEDLANDS, WESTERN AUSTRALIA, 6009; ²DEPARTMENT OF LABORATORY MEDICINE, DIVISION OF CLINICAL IMMUNOLOGY, KAROLINSKA HOSPITAL, S-17176 STOCKHOLM, SWEDEN; AND ³DEPARTMENT OF MEDICINE, THE UNIVERSITY OF WESTERN AUSTRALIA, QE II MEDICAL CENTRE, NEDLANDS, WESTERN AUSTRALIA, 6009

ABSTRACT. Extracts of *Lepidoglyphus destructor* were examined for the presence of digestive enzymes known to be allergenic in the pyroglyphid mites, such as *Dermatophagoides pteronyssinus* and *D. farinae*, with particular emphasis on the proteases and carbohydrases. Three serine proteases and one cysteine protease were detected, each with an apparent molecular weight of 25 K as judged by gel filtration. The serine proteases appeared to correspond to the trypsin, chymotrypsin and collagenolytic enzymes previously demonstrated in mites belonging to the genus *Dermatophagoides*. Chromatofocusing studies showed that each of the serine proteases was polymorphic. Extracts of *L. destructor* were also found to contain amylase, glucoamylase and an enzyme(s) that lysed Gram-positive bacteria, such as *Micrococcus lysodeikticus* and *Bacillus megatarium*. These data indicate that extracts of *L. destructor* contain a spectrum of digestive enzymes similar to that shown to be present in the Pyroglyphid mites. The allergenicity of such enzymes in *L. destructor* remains to be determined. COMP BIOCHEM PHYSIOL 119B;2:341–347, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. Allergens, trypsin, chymotrypsin, bacteriolysins, amylase, glucoamylase, proteases, storage mites

INTRODUCTION

Allergy to the nonpyroglyphid storage mites represents a significant occupational disease in rural communities and is associated with both upper and lower respiratory tract symptoms (7). It has been shown to be important in many countries including Canada, Sweden, Denmark, the United Kingdom, and Finland [reviewed in (25,26)]. The major mite species responsible for sensitisation are *Lepidoglyphus destructor* and *Glycyphagus domesticus* from the family Glycyphagidae, and *Acarus siro* and *Tyrophagus putrescentiae* from the family Acaridae. Of these, particular attention has been devoted to the study of *L. destructor* in an attempt to characterise the most significant allergens, and at least four allergens, with apparent molecular weights (Mol. wt) of 15 K, 39 K, 53 K, and 79 K, have been shown to be important (1,14,24,28). However, the 15 K allergen is the most commonly recognised since it occurs with a frequency of 85–90% of *L. destructor* allergic individuals (9,10,24,28).

The immunochemical relationship between storage mite allergens and other clinically important mites has also been studied in some detail in order to determine the degree of

crossreactivity (9). Data from several studies indicate that the degree of allergenic crossreactivity between *L. destructor* and the pyroglyphid dust mites belonging to the genus *Dermatophagoides* is limited. However, despite this lack of immunological crossreactivity, studies indicate that the 15 K allergen originally designated Lep d 1 is structurally related to the group 2 pyroglyphid allergens (17,27), and its designation has recently been changed to reflect this finding (16). These data suggest that biochemically similar allergens are present in most mite species, but share limited sequence identity.

Recent studies from our laboratory and elsewhere have shown that several of the major allergens from mites such as *D. pteronyssinus* and *D. farinae* are enzymes associated with mite digestive processes, since they are present in faecal extracts. They are predominantly hydrolytic enzymes and include the group 1 cysteine proteases (5), the group 3 trypsins (23), the group 4 amylases (13), the group 6 chymotrypsins (29) and the group 9 cathepsin G-like serine proteases (12). Other enzymes, such as a bacteriolytic enzyme(s), glucoamylase, and aminopeptidase (18,19) have also been demonstrated in faecal extracts, but their allergenicity has yet to be determined. These data suggest that a similar spectrum of enzymes will be present in mites belonging to other genera. We have, therefore, initiated studies to determine whether hydrolytic enzymes corresponding to those described above for *Dermatophagoides* spp. are present in the

Address reprint requests to: Geoffrey A. Stewart, Department of Microbiology, The University of Western Australia, QE II Medical Centre, Nedlands, Western Australia, 6009. Tel. (61-8) 9346 3915; Fax (61-8) 9346 2912; E-mail: geoffrey@cyllene.uwa.edu.au

Received 23 June 1997; revised 28 October 1997; accepted 30 October 1997.

storage mite. The data obtained indicated that all of the previously described hydrolytic enzymes can be detected in *L. destructor*.

MATERIALS AND METHODS

Mite Extracts and Chemicals

Combined whole mite and faecally enriched spent growth media from *D. pteronyssinus* and *D. farinae* media were obtained from Commonwealth Serum Laboratories (Parkville, Australia), whereas that for *L. destructor* was obtained from Allergon (Ängelholm, Sweden). Mite extracts were prepared by extracting samples in either 0.01 M phosphate buffer, pH 7.2, containing 0.15 M NaCl (PBS) for protease, glucoamylase, and amylase analyses, or 0.01 M phosphate buffer, pH 6.2, containing 50 mM EDTA and 2 mM dithiothreitol (DTT) for bacteriolytic enzyme analyses (18). Control extracts were also prepared from unused growth medium. After extraction and centrifugation, mite enzymes were assayed without further processing or after precipitation with alcohol and reconstitution in appropriate buffers or after dialysis against appropriate buffers prior to enzyme assay. General chemicals, synthetic protease substrates were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.) except where otherwise stated.

Determination of Protein Concentration

The protein concentrations of individual samples were determined using the Lowry modification of the Folin assay as described (15) and bovine serum albumin as the standard.

Determination of Enzyme Activity

NONSPECIFIC PROTEASE. Serine and cysteine protease activities were determined using a nonspecific, insoluble diazotised collagen substrate (Azocoll, Calbiochem, La Jolla, CA, U.S.A.) as previously described (18,21). Cysteine protease activity was determined using samples previously reduced with DTT, and activity calculated by subtracting that obtained in the absence of DTT from that obtained in its presence. The results were expressed in units (U) where

1 U represented the optical density (OD) $\times$ 1000/min/mg protein.

Trypsin, Chymotrypsin and Collagenolytic Enzymes

Specific trypsin activity was determined using N-benzoyl-L-arginine-p-nitroanilide (BApNA), whereas chymotrypsin and collagenolytic enzyme activities were determined using succinyl-alanyl-alanyl-prolyl-phenylalanyl-p-nitroanilide (SA₂PFpNA) and succinyl-alanyl-alanyl-prolyl-leucyl-p-nitroanilide (SA₂PLpNA) as described (12,20). Assays were performed in microtitre trays and data expressed as the amount of substrate hydrolysed in nmoles/min/mg protein, where an increase in optical density of 0.1 in the assay corresponded to 36.1 nmoles of substrate converted.

Bacteriolytic Activity

Activity was determined using a modification of the *Micrococcus lysodeikticus* lysis assay described previously (18,21). In brief, 50 μ l of test sample were added to 200 μ l of bacterial suspension (200 μ g/ml in 0.01 M phosphate buffer, pH 6.2, containing 500 μ g/ml bovine serum albumin and 2 mM DTT) in 96-well microtitre plates. The OD at 450 nm was determined at the beginning of the assay and the plate incubated at room temperature for 12 min. The decrease in OD was then determined and the data expressed in U, where 1 U represented the decrease in OD $\times$ 1000/min/mg protein. Experiments were also performed using *Bacillus megatarium* as an indicator of lysis.

Amylase

Amylase activity was assessed using agarose containing 0.25% (w/v) soluble potato starch as described previously and results expressed in either U/mg or U/ml (2,13).

Glucoamylase

Glucoamylase activity was determined by measuring the release of glucose from 2% (w/v) soluble starch as described previously (18). The glucose released was determined by the

TABLE 1. Quantitative analyses of general and specific protease activity present in extracts of *Dermatophagoides pteronyssinus*, *D. farinae*, and *Lepidoglyphus destructor*

Extract	General and specific protease activity				
	Serine protease*	Cysteine protease*	BApNA†	SA ₂ PFpNA†	SA ₂ PLpNA†
<i>D. pteronyssinus</i>	12	16	40	113	24
<i>D. farinae</i>	14	8	35	111	26
Control growth medium	0	0	2	0	0
<i>L. destructor</i>	27	19	28	187	29
Control growth medium	0	0	1	5	1

*Activity determined using Azocoll and results expressed as units/min/mg of protein.

†Activity determined using substrates indicated and results expressed as nmol/min/mg protein.

glucose oxidase method using commercial reagents (Trace Scientific, Baulkham Hills, Australia). The results were expressed in either U/mg or U/ml.

Determination of Isoelectric Point

The isoelectric point(s) (pI) of the various enzymes in mite extracts was determined by chromatofocusing. Mite samples were extracted at a concentration of 50 mg/ml in 5 mM Tris-HCl buffer, pH 8.3, for 2 hr at 4°C. The material was dialysed, centrifuged, and the supernatant applied to a column (1 × 17 cm) of diethylaminoethyl cellulose (DE53; Whatman, Maidstone, U.K.) equilibrated with the same buffer. Chromatofocusing was performed over the pH range 3–8.3 as described (18) and appropriate fractions analysed for enzyme activity as described above.

Determination of Apparent Mol. Wt

The apparent Mol. wt of the individual enzymes from *L. destructor* were determined by gel filtration on a 1.2 × 53 cm column of G-75 (Pharmacia, Uppsala, Sweden) equilibrated with PBS. The elution position of each enzyme was determined using the appropriate enzyme assay and the Mol. wt determined from a graph of K_{av} versus log Mol. wt constructed using the Mol. wt markers cytochrome C, α -chymotrypsinogen, and ovalbumin.

RESULTS

Proteases in *L. destructor*

Both serine and cysteine proteases were detected in extracts of *L. destructor* using a general protease substrate (Table 1). There was an approximately 2-fold greater amount of general serine protease activity in *L. destructor* than in the two *Dermatophagoides* spp., although cysteine protease activities were similar. When BApNA substrate was used to detect trypsin, there was a 1.2–1.4-fold greater amount in *Dermatophagoides* spp. than in *L. destructor*. When the SA₂PFpNA substrate was used, there was 1.6-fold more activity in *L. destructor* than in *Dermatophagoides* spp. When the related substrate SA₂PLpNA was used, each species was similar in activity.

The proteases present in *L. destructor* were examined by chromatofocusing and the data compared with those obtained for *D. pteronyssinus* and *D. farinae*. For brevity, only the profiles obtained for *L. destructor* are shown since the data obtained for the pyroglyphid mites were similar to those previously reported (18). The pI spectrum of trypsin-like enzymes in *L. destructor* (Fig. 1) was similar to that obtained with *D. pteronyssinus*, but not that of *D. farinae*, which showed a restricted range of isoforms (Table 2). The spectrum obtained with *L. destructor* using SA₂PFpNA was similar to that seen when SA₂PLpNA was used, with the exception of the most cationic and anionic isoforms. In this

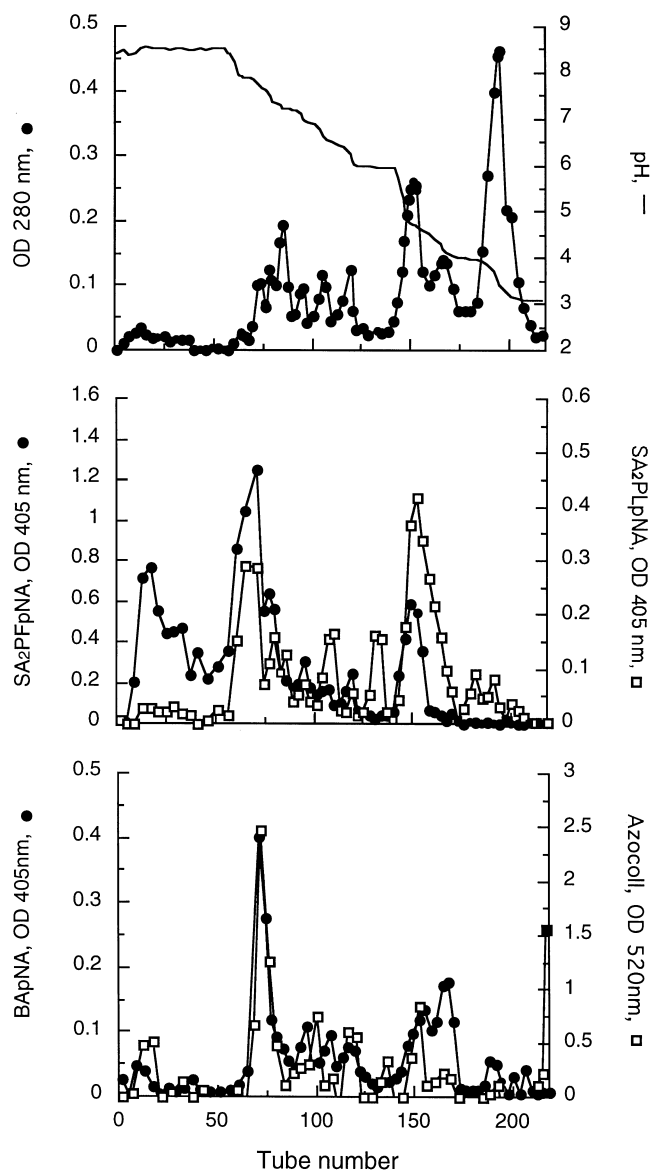


FIG. 1. Chromatofocusing analyses of proteases present in combined whole mite and faecal extract of *Lepidoglyphus destructor*. The top panel shows the relationship between pH and the protein eluate measured at 280 nm; the middle panel shows the elution of proteases detected using SA₂PLpNA and SA₂PFpNA; and the bottom panel shows the relationship between proteases detected using the trypsin substrate BApNA and the general substrate, Azocol.

regard, the cationic protein was more active with SA₂PFpNA, whereas the anionic protein was more active with SA₂PLpNA. Similar results were obtained with both *D. pteronyssinus* and *D. farinae*.

Gel filtration analyses of the proteases in *L. destructor* extract (Fig. 2) and *Dermatophagoides* spp. (data not shown) were also performed. These studies demonstrated the presence of both general protease and cysteine protease activity using azocol as substrate. The general protease activity (e.g., without DTT activation) indicated the presence of a

TABLE 2. Serine protease isoforms in extracts of *Dermatophagoides pteronyssinus*, *D. farinae*, and *Lepidoglyphus destructor*

Substrate	No. of major isoforms			pI of major isoforms		
	<i>D. pteronyssinus</i>	<i>D. farinae</i>	<i>L. destructor</i>	<i>D. pteronyssinus</i>	<i>D. farinae</i>	<i>L. destructor</i>
BAPNA	9 (8)	2 (14)	3 (8)	4 to >8	5, 5.1	4.1, 4.6, 7.0
SA ₂ PFpNA	2 (4)	2 (0)	3 (6)	3.8, 4.4	3.7, 4.1	4.8, 7.8, >8.0
SA ₂ PLpNA	2 (3)	3 (5)	2 (12)	4.5, >8	3.8, 4.2, >8	4.6, 7.9

Isoforms detected by chromatofocusing. Figures in parentheses represent the number of minor isoforms detected, as judged empirically.

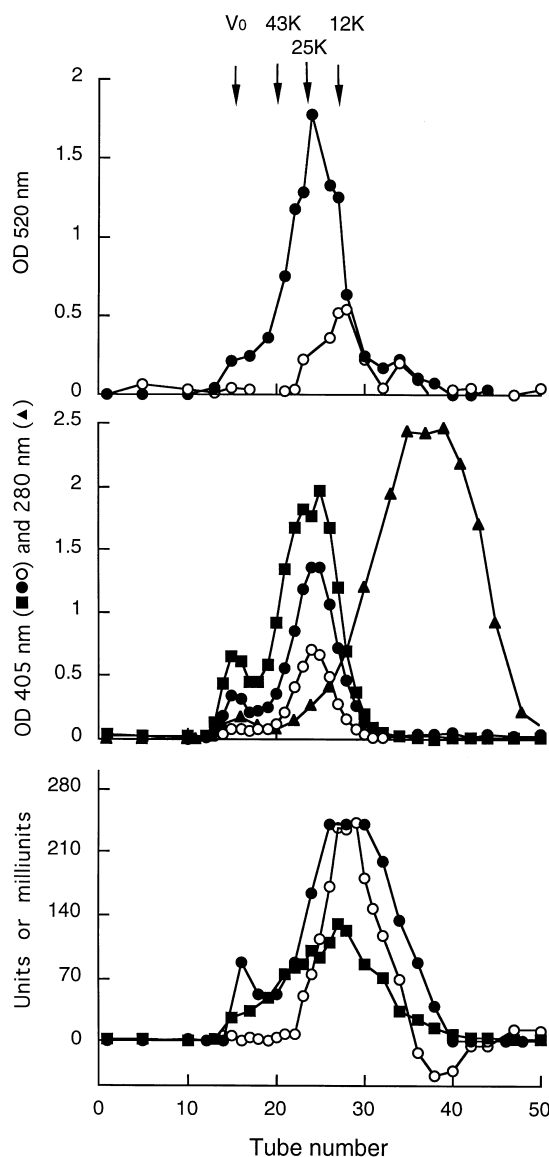


FIG. 2. Sephadex G-75 gel filtration analysis of enzymes from combined whole mite and faecal extract of *Lepidoglyphus destructor*. The top panel shows the elution profiles of general serine protease (●) and cysteine protease activity (○) detected using azocoll substrate; the middle panel shows the elution profiles of proteases detected using BAPNA (○), SA₂PLpNA (●) and SA₂PFpNA (■) and protein measured at 280 nm (▲); the bottom panel shows the elution profiles of amylase (●), glucoamylase (■) and bacteriolytic activity (○). The data for glucoamylase and lysozyme are expressed in milliU/ml.

minor peak eluting in the void volume and a major peak of activity eluting in a similar position to that of α -chymotrypsinogen (Fig. 2) with an apparent Mol. wt of 25 K. The cysteine protease eluted in a similar position to that of cytochrome C. Analyses performed using specific synthetic substrates yielded similar results to those obtained with azocoll under nonreducing conditions (Fig. 2).

Amylase and Glucoamylase in *L. destructor*

Both glucoamylase and amylase activities were detected in all three mite species (Table 3). *D. farinae* and *L. destructor* contained the greatest concentrations of amylase activity whereas *D. farinae* and *D. pteronyssinus* contained the greatest concentrations of glucoamylase activity. A number of isoforms were detected by chromatofocusing (Fig. 3) and a similar spectrum was obtained for each enzyme. In contrast to the equivalent enzymes from *D. pteronyssinus* and *D. farinae*, the pI of both amylase and glucoamylase from *L. destructor* were slightly more cationic (Table 4). The apparent Mol. wt of amylase was about 10 K, whereas several peaks of glucoamylase activity were detected with apparent Mol. wt of 12, 21, and 37 K (Fig. 2).

Bacteriolytic Enzymes in *L. destructor*

Extracts of *D. pteronyssinus*, *D. farinae* and *L. destructor* were shown to possess bacteriolytic activity using *M. lysodeikticus*, and activity in each mite species was enhanced by treatment with DTT. The degrees of enhancement for *D. pteronyssinus*, *D. farinae*, and *L. destructor* were 2.5-, 3.1- and 1.6-fold, respectively. Chromatofocusing studies of *L. destructor* (Fig. 3) revealed the presence of a number of lytic peaks with pI ranging from 4 to >8.5, although the major peaks of activity were 7.4 and >8.0 (Table 4). These results were similar to those obtained with both *D. farinae* and *D. pteronyssinus* (Table 4). The apparent Mol. wt of the bacteriolytic enzyme(s) in *L. destructor* was about 10 K as judged by gel filtration, although there was evidence of a shoulder on the descending part of the peak, suggesting lower Mol. wt forms (Fig. 2).

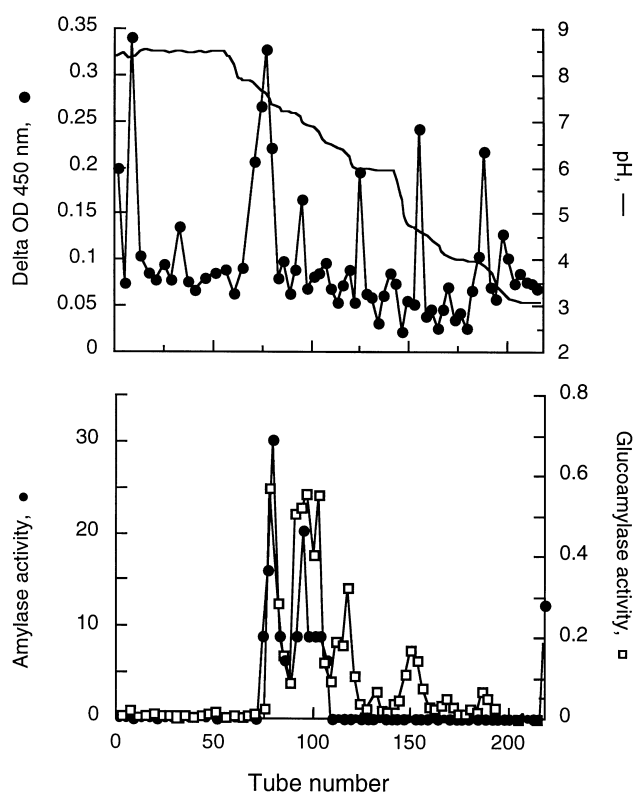
DISCUSSION

The hydrolytic enzymes present in house dust and other storage mites have been studied previously, but from the

TABLE 3. Quantitative analyses of amylase and glucoamylase activities present in extracts of *Dermatophagoides pteronyssinus*, *D. farinae*, and *Lepidoglyphus destructor*

Extract	Carbohydrase activity		Bacteriolytic activity	
	Amylase	Glucoamylase*	<i>Micrococcus lysodeikticus</i> †	<i>Bacillus megatarium</i> †
<i>D. pteronyssinus</i>	149	90	21.3	3.5
<i>D. farinae</i>	222	150	7.0	6.6
Control growth medium	0	0	0	0
<i>L. destructor</i>	194	40	14.5	2.6
Control growth medium	0	0	0	0

Activities expressed as *units/mg or †units/mg/min.

**FIG. 3.** Chromatofocusing analyses of carbohydrases present in combined whole mite and faecal extract of *Lepidoglyphus destructor*. The top panel shows the relationship between pH and bacteriolytic activity detected using *Micrococcus lysodeikticus*; and the bottom panel shows the elution of amylase and glucoamylase.

viewpoint of trophic specialisation. In these studies, mites such as *L. destructor*, *Glycyphagus* spp., *Acarus* spp., and *Tyrophagus* spp. were shown to contain trypsin, chymotrypsin, esterases, lipase, glycosidases, cellulase, amylase, lysozyme, and chitinase (2–4). The data indicated that although the enzymatic profiles obtained for each species varied both qualitatively and quantitatively, they were consistent with known ecological findings. For example, primarily phytophagous mites, such as *Tyrophagus* spp. and *Rhizoglyphus* spp. contained cellulase, an enzyme absent from mites such as *L. destructor* and *A. siro*, which feed on fungi. Similarly, the latter mite species were relatively rich in proteases compared to the former. The data obtained from the study of the enzymes from *L. destructor* described herein are consistent with previous observations regarding the presence of the enzymes described above (2–4,6). However, we have shown that extracts of this mite also contained a glucoamylase-like enzyme (which cleaves terminal α -1,4 and α -1,6 linked glucose units from glucans, such as starch), a cysteine protease, and a collagenolytic enzyme. These, and enzymes such as trypsin and chymotrypsin, have all been shown to be allergenic in Pyroglyphid mites, but it is not yet clear whether any of the *L. destructor* enzymes is allergenic in individuals specifically exposed to the storage mite.

In this study, we examined the storage mite enzymes in some detail to establish their relationships to the corresponding enzymes from the pyroglyphid mites. With regard to the serine proteases, the data obtained indicated that, on the basis of the substrates used and chromatofocusing studies, *L. destructor* contained the three enzymes reported previously for *D. pteronyssinus* (12,18,20) and that each was

TABLE 4. Amylase, glucoamylase, and bacteriolytic isoforms in extracts of *Dermatophagoides pteronyssinus*, *D. farinae*, and *Lepidoglyphus destructor*

Enzyme	No. of major isoforms*			pI of major isoforms		
	<i>D. pteronyssinus</i>	<i>D. farinae</i>	<i>L. destructor</i>	<i>D. pteronyssinus</i>	<i>D. farinae</i>	<i>L. destructor</i>
Amylase	2 (5)	3 (4)	2 (1)	5.6, 5.8	4.4, 5.7	7, 7.4
Glucoamylase	3 (1)	4 (7)	3 (2)	6 to 6.9	4 to 6.2	6.8, 7, 7.6
Bacteriolytic activity	3 (6)	2 (6)	3 (5)	5.8 to >8	7.6, >8	7.4, >8

*Isoforms detected by chromatofocusing. Figures in parentheses represent the number of minor isoforms detected as judged empirically.

polymorphic. Thus, multiple trypsin-like enzymes and multiple enzymes with specificities for SA₂PFpNA and SA₂PLpNA substrates were detected. The latter two substrates detect a variety of enzymes with a specificity for hydrophobic amino acid residues, such as chymotrypsin, elastase, and cathepsin-G, and have been used in our laboratory to identify a novel serine protease allergen from *D. pteronyssinus* designated Der p 9 (12), which was clearly different from mite trypsin and chymotrypsin, although showing a high degree of sequence homology [(12) and unpublished observations]. The Der p 9 protease showed a preference for SA₂PFpNA and was highly cationic (pI of its major isoform was 11.0). It is likely, therefore, that the cationic protease from *L. destructor*, which caused a relatively marked cleavage of SA₂PFpNA, was an analogue of the Der p 9 protease. It also seems likely that, based on data obtained with *D. pteronyssinus* and *D. farinae* (20), the enzyme from *L. destructor* that showed both SA₂PFpNA and SA₂PLpNA cleaving activity with a pI of 4.6–4.8 corresponded to chymotrypsin.

The apparent Mol. wt of the serine proteases detected in *L. destructor* were similar to each other and corresponded to those observed for the pyroglyphid mites. A high Mol. wt peak of activity was also detected with each substrate, but the significance of this finding is unclear. However, it suggests the presence of aggregated material or interaction between protease and other proteins present in the mite extracts. In addition to the serine proteases, cysteine protease activity was also detected in *L. destructor* when whole mite extracts and gel filtration fractions thereof were analysed, but we were unable to detect this activity by chromatofocusing. The relationship between the enzyme(s) mediating this activity and the pyroglyphid group 1 allergens is unclear at present. However, previous data obtained using an inhibition-immunoblotting technique indicated that a group 1 analogue was present not only in *L. destructor* but also in other storage mites including *T. putrescentiae* and *A. siro* (9,11). The apparent Mol. wt of the enzyme was about 12 kDa. This value is similar to that reported for Der p 1 using the same technique, although both values are approximately half that expected on the basis of SDS-PAGE and sequence data (5,21). The reason for the low Mol. wt on gel filtration is unclear.

Amylase and glucoamylase enzymes were also detected in *L. destructor*. Again, both enzymes were judged to be heterogeneous on the basis of charge, since 2–3 amylase isoforms and 5–6 glucoamylase isoforms were detected, although there was some degree of overlap in the positions of amylase and glucoamylase on chromatofocusing. Recent data from our laboratory obtained with *D. pteronyssinus* extracts (21) clearly indicate that amylase and glucoamylase are distinct entities, but the above findings also raise the possibility that either amylase from *L. destructor* breaks down starch to glucose or, alternatively, that the amylase assay used was not specific. Although these possibilities still remain, gel filtra-

tion studies showed marked differences in elution profiles, suggesting the presence of both enzymes in *L. destructor*. In addition, these studies also showed that both putative enzymes eluted with anomalously low apparent Mol. wt, suggesting interaction with glucose-based chromatography media consistent with previously reported data for amylases from other invertebrate species (30) and for glucoamylase and amylase from pyroglyphid mites (13).

In addition to glucoamylase and amylase activities, bacteriolytic activity was also detected in *L. destructor*, in accord with data reported for *D. pteronyssinus* and *D. farinae* (18,21). Bacteriolysis was demonstrated using two Gram-positive bacterial species, with the most susceptible being *M. lysodeikticus*. At present, it is not clear whether the lytic activity detected in both *L. destructor* and pyroglyphid mites can be ascribed to the archetypal type C lysozymes reported for other invertebrates. However, recent unpublished observations from our laboratory indicate that pyroglyphid mites possess a bacteriolytic enzyme that demonstrates limited homology to a number of bacterial proteins one of which, namely γ -D-glutamyl L diamino acid endopeptidase, possesses bacteriolytic activity. Interestingly, both *L. destructor* and pyroglyphid mite lytic activities were enhanced in the presence of DTT, a finding similar to that for the endopeptidase (8). These data suggest that the mite enzymes are endopeptidases also.

In conclusion, data derived from this investigation indicate that extracts of the storage mite *L. destructor* contain a range of carbohydrases and proteases that, in other mites, have been shown to be allergenic. Further studies are required to establish not only whether the *L. destructor* enzymes are allergenic, but also to determine the physicochemical relationship between storage mite enzymes and those from the pyroglyphid mites. Such studies are currently underway. Finally, the data reported here are consistent with the proposition that enzymatic activity of mite allergens may contribute to allergenicity *per se*. In this regard, we have demonstrated that proteases from *D. pteronyssinus* induce mucosal permeability and proinflammatory cytokine release (22), both of which have the potential to modulate immunogenicity.

*This study was supported by the Asthma Foundation of W.A. Inc., the Australian National Health and Medical Research Council, the Australian Research Council, the Swedish Council for Work Life Research, the Swedish Medical Research Council, the Swedish Association Against Asthma and Allergy and the Hesselman Foundation. We thank Ms. Cathy Pope for excellent technical assistance and Mrs. Sylvia German for her computer/typing expertise. We also thank Commonwealth Serum Laboratories (Parkville, Australia) for providing whole *D. pteronyssinus* and *D. farinae* mites, spent growth medium from both mite cultures and unused growth medium.*

References

1. Ansotegui, I.J.; Härfast, B.; Jeddi-Tehrani, M.; Johansson, E.; Johansson, S.G.O.; van Hage-Hamsten, M.; Wigzell, H. Iden-

- tification of a new major allergen of 39 kilodaltons of the storage mite *Lepidoglyphus destructor*. *Immunol. Lett.* 27:127–130; 1991.
2. Bowman, C.E.; Lessiter, M.J. Amylase and esterase polymorphisms in economically important stored product mites (Acari: Astigmata). *Comp. Biochem. Physiol.* 81B:353–360; 1985.
 3. Bowman, C.E. Comparative enzymology of economically important astigmatid mites. In: Griffiths, D.A.; Bowman, C.E. (eds). *Acarology VI*. Chichester: Ellis Horwood; 1984:993–1001.
 4. Childs, M.; Bowman C.E. Lysozyme activity in six species of economically important Astigmatid mites. *Comp. Biochem. Physiol.* 70B:615–617; 1981.
 5. Chua, K-Y; Stewart, G.A.; Thomas, W.R.; Simpson, R.J.; Dilworth, R.J.; Plozza, T.M.; Turner, K.J. Sequence analysis of cDNA coding for a major house dust mite allergen, Der p 1. Homology with cysteine proteases. *J. Exp. Med.* 167:175–182; 1988.
 6. Colloff, M.J. Differences between the allergen repertoires of house dust mites and stored-products mites. *Clin. Immunology* 16:114–123; 1993.
 7. Cuthbert, O.D.; Brostoff, J.; Wraith, D.G.; Brighton, W.D. "Barn allergy": Asthma and rhinitis due to storage mites. *Clin. Allergy* 9:229–36; 1979.
 8. Hourdou, M.L.; Duez, C.; Joris, B.; Vacheron, M.J.; Guinand, M.; Michel, G.; Ghuysen, J.M. Cloning and nucleotide sequence of the gene encoding the γ D-glutamyl-L-diamino acid endopeptidase II of *Bacillus sphaericus*. *FEMS Microbiol. Lett.* 91:165; 1992.
 9. Johansson, E.; Borgå, Å; Johansson, S.G.O.; van Hage-Hamsten, M. Immunoblot multiallergen inhibition studies of allergenic cross-reactivity of the dust mites *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clin. Exp. Allergy* 21: 511–518; 1991.
 10. Johansson, E.; van Hage-Hamsten, M.; Johansson, S.G.O. Demonstration of allergen components in the storage mite *Lepidoglyphus destructor* by an immunoblotting technique. *Int. Arch. Allerg. Appl. Immunol.* 85:8–13; 1988.
 11. Johansson, E.; Johansson, S.G.O.; van Hage-Hamsten, M. Allergenic characterisation of *Acarus siro* and *Tyrophagus putrescentiae* and their crossreactivity with *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clin. Exp. Allergy* 24: 743–751; 1994.
 12. King, C.M.; Simpson, R.J.; Moritz, R.L.; Reid, G.E.; Thompson, P.J.; Stewart, G.A. The isolation and characterisation of a novel collagenolytic serine protease allergen (Der p 9) from the house dust mite, *Dermatophagoides pteronyssinus*. *J. Allerg. Clin. Immunol.* 98:739–747; 1996.
 13. Lake, F.R.; Ward, L.D.; Simpson, R.J.; Thompson, P.J.; Stewart, G.A. House dust mite-derived amylase: Allergenicity and physicochemical characterisation. *J. Allerg. Clin. Immunol.* 87:1035–1042; 1991.
 14. Olsson, S.; Härfast, B.; Johansson, S.G.; van Hage-Hamsten, M. Detection of at least one high-molecular-mass, IgE-binding component of the dust mite *Lepidoglyphus destructor*. *Allergy* 49:620–625; 1994.
 15. Peterson, G.L. Determination of total protein. *Meth. Enzymol.* 91:95–119; 1983.
 16. Platts-Mills, T.A.E.; Thomas, W.R.; Aalberse, R.C. *et al.* Dust mite allergens and asthma: Report of a second international workshop. *J. Allerg. Clin. Immunol.* 89:1046–1060; 1992.
 17. Schmidt, M.; Olsson, S.; van der Ploeg, I.; van Hage-Hamsten, M. cDNA analysis of the mite allergen Lep d 1 identifies two different isoallergens and variants. *FEBS Lett.* 370:11–14; 1995.
 18. Stewart, G.A.; Bird, C.H.; Krska, K.D.; Colloff M.J.; Thompson, P.J. A comparative study of allergenic and potentially allergenic enzymes from *Dermatophagoides pteronyssinus*, *D. farinae* and *Euroglyphus maynei*. *Exp. Appl. Acarol.* 16:165–180; 1992.
 19. Stewart, G.A.; Butcher, A.; Lees, K.; Ackland, J. Immunological and enzymatic analyses of extracts of the house dust mite *Dermatophagoides pteronyssinus*. *J. Allerg. Clin. Immunol.* 77:14–24; 1986.
 20. Stewart, G.A.; Kollinger, M.R.; King, C.M.; Thompson, P.J. A comparative study of three serine proteases from *Dermatophagoides pteronyssinus* and *D. farinae*. *Allergy* 49:553–560; 1994.
 21. Stewart, G.A.; Lake, F.R.; Thompson, P.J. Faecally derived hydrolytic enzymes from *Dermatophagoides pteronyssinus*—physicochemical characterisation of potential allergens. *Int. Arch. Allerg. Appl. Immunol.* 95:248–256; 1991.
 22. Stewart, G.A.; Thompson, P.J.; McWilliam, A.S. Biochemical properties of aeroallergens: Contributory factors in allergic sensitization. *Pediatr. Allerg. Immunol.* 4:163–172; 1993.
 23. Stewart, G.A.; Ward, L.D.; Simpson, R.J.; Thompson, P.J. The group III allergen from the house dust mite *Dermatophagoides pteronyssinus* is a trypsin-like enzyme. *Immunology* 75: 29–35; 1992.
 24. van Hage-Hamsten, M.; Bergman, T.; Johansson, E.; Persson, B.; Jörnvall, H.; Härfast, B.; Johansson, S.G.O. N-Terminal amino acid sequence of principle allergen of storage mite *Lepidoglyphus destructor*. *Lancet* 340:614; 1992.
 25. van Hage-Hamsten, M.; Johansson, S.G.O. Storage mites. *Exp. Appl. Acarol.* 16:117–128; 1992.
 26. van Hage-Hamsten, M. Allergens of storage mites. *Clin. Exp. Allergy* 22:429–431; 1992.
 27. Varela, J.; Ventas, P.; Carreira, J.; Barbas, J.A.; Gimenez-Gallego, G.; Polo, F. Primary structure of Lep d I, the main *Lepidoglyphus destructor* allergen. *Eur. J. Biochem.* 225:93–98; 1994.
 28. Ventas, P.; Carreira, J.; Polo, F. Identification of IgE-binding proteins from *Lepidoglyphus destructor* and the production of monoclonal antibodies to a major allergen. *Immunol. Lett.* 29: 229–234; 1991.
 29. Yasueda, H.; Mita, H.; Yui, Y.; Shida, T. Isolation and characterisation of two allergens from *Dermatophagoides farinae*. *Int. Arch. Allerg. Appl. Immunol.* 81:214–223; 1986.
 30. Wojtowicz, M.B.; Brockerhoff, H. Isolation and some properties of the digestive amylase of the American lobster (*Homarus americanus*). *Comp. Biochem. Physiol.* 42B:295–302; 1972.