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**House dust mite-derived amylase:
Allergenicity and
physicochemical characterization**

Fiona R. Lake, FRACP,* ** ** Larry D. Ward, PhD,***
Richard J. Simpson, PhD,*** Philip J. Thompson, FRACP,** and
Geoffrey A. Stewart, PhD* Perth and Melbourne, Western Australia**

Amylase activity was found in extracts of both Dermatophagoides pteronyssinus whole mite (0.16 U/mg) and spent growth medium (0.01 U/mg) but not in unused growth medium. It was also detected in all extracts of house dust obtained from mattresses (n = 20; geometric mean, 1.95 U/gm) and in 18 extracts of dust obtained from lounge room carpets (n = 20; geometric mean, 0.54 U/gm). Although the origins of amylase in dust are unclear, enzyme activity correlated with mite counts (n = 40; r = 0.35; p < 0.05) and Der p I concentrations (r = 0.41; p < 0.01). Mite amylase was purified from spent growth medium by affinity chromatography, gel filtration, and chromatofocusing. It was physicochemically similar to mammalian amylase with regard to molecular weight (60,000), charge heterogeneity (isoelectric point, 5 to 7) and the capacity to bind to an organomercurial affinity matrix. The optimum pH for enzymatic activity was revealed to be 6.4. IgE immunoblot studies demonstrated that the enzyme was allergenic and that its expression was dependent on the integrity of intrachain disulfide bonds. Sera from 25% of mite-allergic children and 46% of mite-allergic adults contained specific IgE to mite amylase. IgE to amylase was associated (p < 0.01) with increased concentrations of total mite-specific IgE determined with a direct RAST assay. (J ALLERGY CLIN IMMUNOL 1991;87:1035-42.)

From *The Western Australian Research Institute for Child Health, Princess Margaret Hospital, Perth, **Department of Medicine, University of Western Australia, Perth, and ***Joint Protein Structure Laboratory, Ludwig Institute for Cancer Research and Walter and Eliza Hall Institute, Melbourne, Australia.

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Reprint requests: G. A. Stewart, PhD, The Western Australian Research Institute for Child Health, Princess Margaret Hospital, Thomas St., Subiaco, Perth, Western Australia 6008.

Fiona R. Lake, FRACP, is a recipient of a Western Australia and M. G. Saw Fellowship from the University of Western Australia.

****Current address: National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson St., Denver, CO 80206.

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Allergy to the house dust mites *Dermatophagoides pteronyssinus* and *D. farinae* is associated with asthma, rhinitis, and atopic dermatitis, and these observations have stimulated many studies into the physicochemical nature of the allergens involved.¹ Qualitative studies have demonstrated that the number of allergens detected varies with the method used. Thus, crossed radioimmuno-electrophoretic analyses have revealed the presence of up to 19 allergens,²⁻⁴ whereas immunoblotting studies performed after SDS-PAGE indicated that as many as 32 components may be allergenic.^{5, 6}

Two of the major allergens, *Der p* I and II, have been studied in most detail with both physicochemical and, more recently, recombinant DNA technology. With regard to the latter, both allergens have been cloned and sequenced.^{7, 8} Analysis of these data and subsequent biochemical studies have demonstrated *Der p* I to be a cysteine proteinase.^{7, 9} Similar studies on data derived by conventional protein sequencing of the allergens designated *Der p* III and *Der f* III^{9, 10} have demonstrated that both are also proteinases⁹ but, unlike *Der p* I, belong to the serine group of proteinases. The findings that these allergens are present in fecally enriched mite extracts^{9, 11} suggests that the proteinases are intimately involved in mite digestion and that other gut enzymes may also be allergenic.

A variety of mite digestive enzymes have been described in studies primarily designed to determine the degree of trophic specialization among economically important mites, such as the storage mites, or in determining the anatomic structure of the mite digestive system per se. Thus, mites, such as *Glycophagus* spp, *Acarus* spp, and *Tyrophagus* spp, have been demonstrated to contain proteinases, esterases, lipase, glycosidases, cellulase, amylase, lysozyme, and chitinase.¹² Similar studies have been performed with the house dust mites *D. pteronyssinus* and *D. farinae*, but in only one study has the possible allergenicity of the nonproteinase mite enzymes been addressed. In this regard, esterase, phosphatase, aminopeptidase, and glycosidase were demonstrated to be devoid of allergenic activity.⁴

It is not known whether the remaining mite digestive enzymes are allergenic, but such analyses appear warranted, particularly if the enzymes can be demonstrated in particles such as fecal pellets that are capable of being inhaled. Amylase has recently been demonstrated to be present in SGM,¹³ and a mite allergen with its expected MW has been demonstrated in immunoblotting studies.^{6, 14} Furthermore, fungal amylase appears to be allergenic and has been reported to be important in triggering occupational asthma.^{15, 16} Consequently, it appeared appropriate to

Abbreviations used

DTT:	Dithiothreitol
HD:	House dust
HDM:	<i>Dermatophagoides pteronyssinus</i>
MW:	Molecular weight
pI:	Isoelectric point
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SGM:	Spent growth medium
UGM:	Unused growth medium

pursue the isolation of HDM amylase and assess its allergenicity.

MATERIAL AND METHODS

Mite extracts and patient sera

Extracts of HDM, SGM, and UGM were prepared as described previously.¹³ HD samples were obtained from mattresses and lounge room carpets from subjects with asthma and control subjects. The mite and *Der p* I concentrations in the HD have been previously reported.¹⁷ Sera were obtained from adult patients attending the Sir Charles Gairdner Hospital and from children attending the Princess Margaret Hospital. The mite-allergic status of patients was determined by skin testing or by RAST. Control sera included sera from known mite allergy-negative subjects, cord serum, and a serum from a subject with hyper-IgE syndrome (500 U/ml). Most sera were also semiquantitatively assessed for mite-specific IgE by RAST with a standard curve constructed from a pool prepared from sera of mite-allergic individuals, and the results were expressed in units per milliliter.

Analytic methods

Amylase analysis. Amylase activity in the original HDM, SGM, UGM, HD, and the purified amylase fractions was measured with a commercial, insoluble, dyed starch substrate (Phadebas; Pharmacia Diagnostics, Sydney, Australia) according to the manufacturer's instructions. The activity was expressed as units per milligram of protein or units per gram of fine dust. Amylase activity in column eluates was assessed by the agarose-starch method as described,¹⁸ and the results were expressed in units per liter of sample or units per milligram of protein after reference to a standard curve constructed with SGM.

pH Optimum. The pH optimum of mite amylase was determined with SGM dissolved in buffers over the pH range 3.4 to 10.2 and the commercial assay.

Gel filtration. Mite extracts were fractionated by gel filtration on Sephadex G-75 (Pharmacia). The apparent MW of amylase in the various extracts were determined as described.¹³ Appropriate fractions were pooled, dialyzed, and freeze-dried. The protein content of extracts and fractions was determined as described.¹⁹

Chromatofocusing. Chromatofocusing was performed at room temperature as described.¹³ Proteins were eluted over

the pH range 3.5 to 8, and firmly bound material was eluted with 1 mol/L of NaCl. Appropriate fractions were pooled, dialyzed, and freeze-dried.

Organomercurial affinity chromatography. Affinity chromatography was performed as described¹³ with SGM. Bound material was desorbed with 40 mmol/L of cysteine HCl (Sigma Chemical Co., St. Louis, Mo.), and fractions were examined for amylase activity as described above.

SDS-PAGE. SDS-PAGE analysis was performed essentially as described²⁰ with either 10% (wt/vol) homogeneous gels or 8% to 18% (wt/vol) gradient gels. Proteins were examined after reduction with DTT or without reduction. Gels were stained with Coomassie brilliant blue R-250 (Sigma Chemical Co.). The apparent MW of the proteins was determined by reference to a standard curve constructed by plotting the log MW of marker proteins (Sigma Chemical Co. or Bio-Rad Laboratories, Richmond, Calif.) against relative mobilities.

IgE immunoblot analysis. Immunoblot analyses were performed as described²¹ with purified amylase and sera diluted 1:10 with 0.01 mol/L of phosphate buffer, pH 7.2, containing 0.15 mol/L NaCl, 0.005% (vol/vol) Tween 20, and 0.5% (wt/vol) bovine serum albumin (CSL, Parkville, Australia). IgE binding was detected with an ¹²⁵I-radiolabeled mouse monoclonal anti-IgE.

N-terminal sequence analysis. The N-terminal sequence of mite-derived amylase was determined after SDS-PAGE and electrotransfer of the allergen from a 12.5% (wt/vol) polyacrylamide gel to polyvinylidene difluoride membrane, as described previously.^{22, 23} The N-terminal sequence obtained was compared with sequences in the Protein Information Resource data bank of the National Biomedical Research Foundation with a commercial computer searching program (MacVector; IBI, New Haven, Conn.).

Purification of mite amylase

Mite amylase was isolated from SGM with the ethanol-glycogen precipitation method.²⁴ A 50% saturated ammonium sulfate precipitate of SGM was dialyzed against distilled water and ethanol and then added at 4° C to a final concentration of 40% (vol/vol). After incubation for 1 hour, the resulting precipitate was centrifuged and discarded. To the supernatant was added 0.06 vol of 12.5% (wt/vol) of oyster glycogen (type II, Sigma Chemical Co.), and the sample was centrifuged. The glycogen-amylase precipitate was washed twice with 0.01 mol/L of phosphate buffer, pH 8.0, containing 40% (vol/vol) ethanol, and the precipitate was dissolved in phosphate buffer and incubated at room temperature for 2 hours to facilitate digestion of the glycogen. The amylase was purified by sequential gel filtration and chromatofocusing.

RESULTS

Detection of amylase activity in mite and HD extracts

Amylase activity was detected in extracts prepared from HDM, SGM, and HD with the commercial assay. It was present in both HDM (0.16 U/mg of pro-

tein) and SGM (0.01 U/mg), but not in UGM. Amylase activity was found in 20 (100%) and 18 (90%) of the 20 mattress and 20 lounge carpet samples analyzed, respectively. The geometric mean amylase activity in the mattress dust (1.95 U/gm of fine dust; range, 0.6 to 1.5) was not significantly greater than that found in the lounge carpet dust (1.83 U/gm; range, 0 to 4.4). Weak but significant correlations between amylase and both total mite counts ($r = 0.35$; $p < 0.05$) and *Der p 1* concentrations ($r = 0.41$; $p < 0.01$) were observed. The correlation between mite counts and immunochemically determined concentrations of *Der p 1* was also significant ($r = 0.75$; $p < 0.001$).

Characterization of mite amylase in HDM and SGM mite extracts

That the MW distributions of amylase activity in both types of mite extracts, as demonstrated by Sephadex G-75 gel filtration, were dissimilar in that two peaks of activity were present in HDM and only one peak was present in SGM is illustrated in Fig. 1. The apparent MW of the two HDM amylase peaks were 10,000 (the major component) and 66,000, whereas the major peak in SGM was 51,000. Amylase in SGM demonstrated marked charge heterogeneity, with most activity in the pI range 5 to 7. Studies with a variety of amylases from other sources have demonstrated that they possess free sulfhydryl groups,²⁵ and mite amylase was demonstrated to bind to the organomercurial-Sepharose 4B matrix. The optimum pH for amylase activity was 6.4.

Isolation of amylase from SGM

Initial attempts were made to isolate amylase by affinity chromatography with a variety of ligands, such as wheat-kernel amylase inhibitor and starch, but these approaches failed (data not presented). However, amylase was successfully isolated with glycogen precipitation. It was initially isolated from SGM and demonstrated, after digestion of the glycogen component by incubating the complex at room temperature for 2 hours, to be allergenic by direct RAST analysis with a serum pool from mite-allergic children. The preparation was demonstrated to be allergenic, but SDS-PAGE analysis demonstrated that the material was impure (data not presented).

A different isolation approach was, therefore, developed that involved the initial ammonium sulfate precipitation step and further purification of the glycogen precipitate by gel filtration and chromatofocusing. Although most amylase was lost at this stage because of insolubility in the alcohol, sufficient amylase was isolated for study, and representative yields

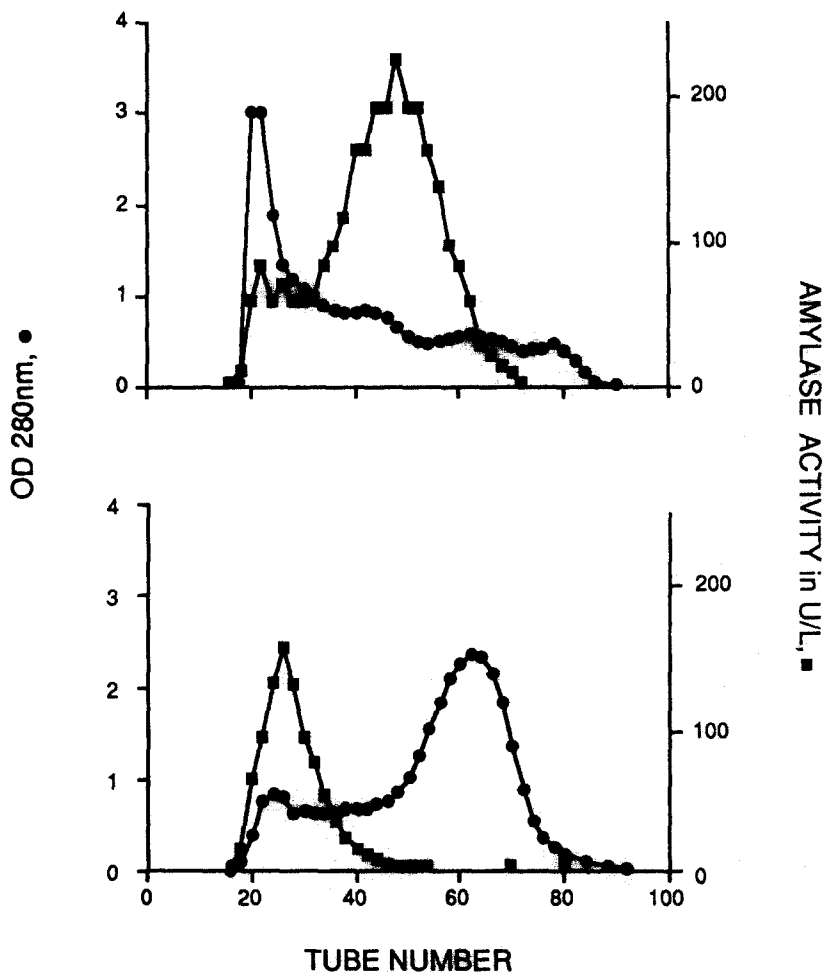


FIG. 1. Gel-filtration analyses of amylase in whole mite extract (**top panel**) and SGM (**bottom panel**) on a column of G-75 (0.9 × 100 cm) equilibrated with 0.02 mol/L of phosphate buffer, pH 6.9, containing 0.006 mol/L of NaCl at 4° C. The column eluate was analyzed for protein by absorption at 280 nm and for enzyme activity with the agarose-starch assay.

of enzyme obtained up to and including the glycogen step are presented in Table I. Yields at subsequent stages are not reported because of the heterogeneity demonstrated at both the gel filtration and chromatofocusing steps. The complexity (as judged by SDS-PAGE after prior reduction of the enzyme) of the various fractions obtained at different stages of purification is illustrated in Fig. 2.

The apparent MW of mite amylase was demonstrated to be 56,000 and 60,000, as judged by 8% to 18% (wt/vol) gradient gel SDS-PAGE analyses performed under nonreducing and reducing conditions, respectively (Fig. 2). Slightly higher values were obtained on 10% (wt/vol) homogeneous gels, namely, 59,000 and 63,000, respectively. One minor, low MW contaminating protein was detected in the purified amylase preparation, but this component was not allergenic (see below).

The amylase fraction obtained after chromatofoc-

using was subjected to SDS-PAGE, electrotransferred to a polyvinylidene difluoride membrane, and sequenced. The *N*-terminal sequence is presented in Table II, together with the *N*-terminal sequence of amylase from *Aspergillus oryzae*.²⁶ Searches with the Protein Information Resource of the National Biomedical Research Foundation failed to reveal homology with any protein sequence.

IgE immunoblot with purified amylase

Purified amylase was found to be allergenic, as judged by immunoblot analysis, but its expression was markedly diminished if immunoblotting was performed after reduction of the enzyme with DTT (Fig. 2). Subsequent immunoblots were, therefore, performed without prior reduction. Sera from 27 mite-allergic adults and 20 children were studied for the capacity of the sera to bind to amylase. Some of these data obtained with these sera, together with appropri-

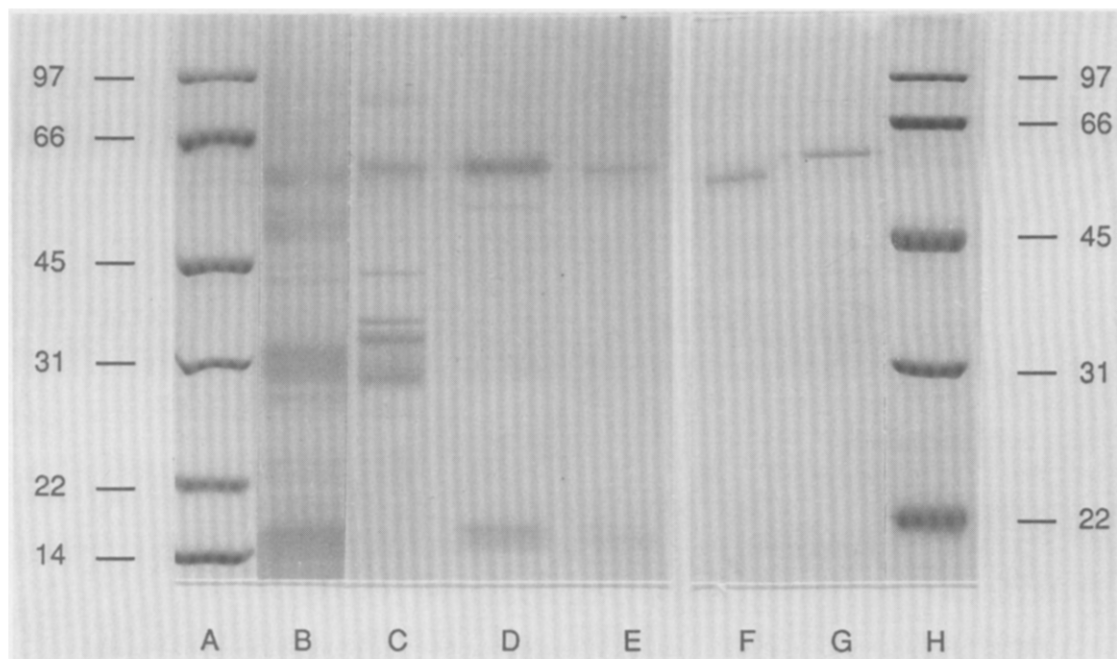


FIG. 2. SDS-PAGE analysis of fractions obtained at various stages involved in the isolation of mite amylase and the influence of reduction on purified amylase; *lane A*, MW markers (in kilodaltons); *lane B*, SGM; *lane C*, the amylase fraction precipitated with glycogen; *lane D*, amylase purified by gel filtration; *lane E*, amylase after gel filtration and chromatofocusing; *lane F*, nonreduced amylase; *lane G*, reduced amylase; and *lane H*, MW markers. Samples in *lanes A* to *E* were analyzed on a different gel than samples illustrated in *lanes G* to *H*.

TABLE I. Yield of amylase at various stages of purification from SGM

Fractionation step	Amylase (total U)	Protein (mg)	Specific activity (U/mg)	Yield (%)
Original SGM	141	1206	0.12	100
Ammonium sulfate precipitation	119	182	0.65	84
Glycogen precipitation	11	1.8	6.10	8

ate controls, are illustrated in Fig. 3. The degree of IgE binding varied between the subjects, ranging from the absence of binding to intense radiostaining. Forty-six percent of the sera from adults and 25% of the sera from children demonstrated IgE binding to mite-derived amylase. In contrast, sera from cord blood, normal nonatopic control subjects, and a subject with hyper-IgE syndrome did not bind. The geometric mean concentration of HDM-specific IgE in sera from those adults that reacted (25 U/ml) was significantly greater ($p = 0.008$) than the mean concentration in sera of adults that did not react with amylase (5 U/ml), as judged by the Student's t test. Similar analyses with children's sera were not performed.

DISCUSSION

Alpha amylases (enzyme commission No. 3.2.1.1) are ubiquitous endoglycosidases that hydrolyze 1,4-

TABLE II. N-terminal sequence of mite amylase

Amylase source	Sequence
	1 to 19
Mite	K Y X N P H F I G X R S V I T X L M E
<i>A. oryzae</i> *	A T P A D W R S Q S I Y F L L T D R F

**A. oryzae* sequence data from reference 26.

glucosidic linkages in polysaccharidases containing three or more 1,4-linked D-glucose units. In mites, amylases are secreted by the salivary glands and stomach to aid in the digestion of starch or glycogen derived from food, such as skin scales, fungi, and bacteria. The presence of amylase in whole body extracts from several genera of mites has been previously reported,¹²

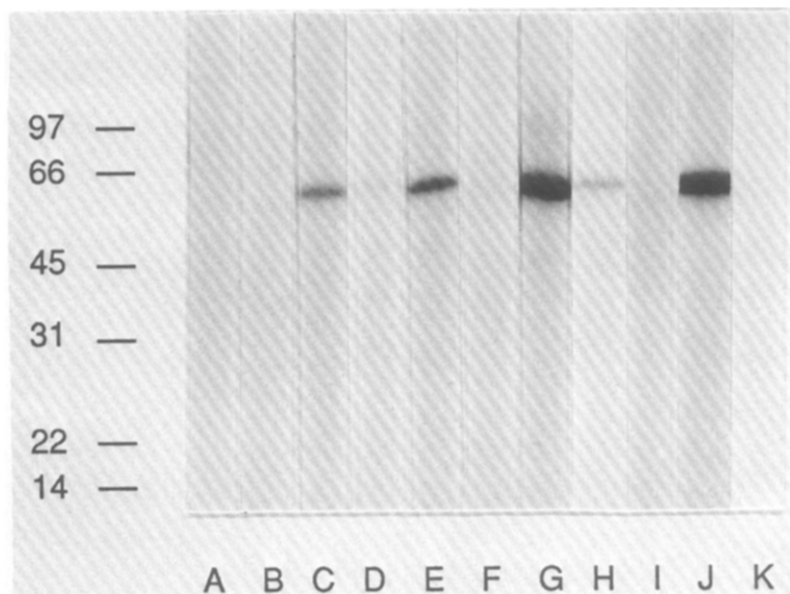


FIG. 3. IgE immunoblot analysis of individual sera from mite-allergic individuals with amylase in the unreduced state; *lane A*, serum from patient with hyper-IgE syndrome; *lane B*, cord serum; *lane C*, serum pool from mite-allergic children; and *lanes D to K*, individual sera.

but detailed physicochemical descriptions of the enzyme per se have been lacking. We have demonstrated in this study that the enzyme was present in extracts prepared from both whole bodies and fecal material from *D. pteronyssinus* and that it was allergenic. In addition, it was also found in mattress and carpet dust, but it was not possible to determine whether it was mite derived, despite significant associations with known correlates of mite infestation.

Amylases from diverse sources have been isolated on the basis of their abnormal behavior on gel filtration matrices comprising glucose polymers, such as Sephadex.²⁷ In our studies, the enzyme eluted at a position equivalent to a molecule with an apparent MW of approximately 13,000 rather than expected MW of about 60,000 because of an enzymatic interaction with the column. In contrast, initial attempts to isolate mite amylase from SGM exploiting this phenomenon failed since this anomalous behavior was not observed. The expected behavior was, however, observed with amylase present in HDM extracts, suggesting the amylase in SGM may have been complexed with an unidentified component of the growth medium, which would be consistent with the known propensity of the enzyme to bind to a variety of polysaccharide-containing components.²⁷ Amylase was, however, successfully purified with the glycogen precipitation method, although the yield was low.

The biochemical characteristics of mite amylase are

in keeping with characteristics described for the enzyme derived from both vertebrate and invertebrate sources. Characteristics include heterogeneity with regard to charge, the possession of free sulfhydryl groups, and an apparent MW of about 60,000.²⁵ The range of pI values reported here for mite amylase is similar to the range reported for the enzyme in storage mites (pI, 4.9 to 7.1) that was determined with thin-layer polyacrylamide isoelectrofocusing.²⁸ The *N*-terminal amino acid sequence obtained for mite amylase did not demonstrate homology with any previously sequenced amylase or other protein sequence. The *N*-termini of amylases from a variety of sources demonstrate little homology with one another; thus, our findings were not unexpected.

IgE immunoblot analyses without prior reduction demonstrated that amylase was allergenic with a frequency of reactivity of 46% with sera from adult patients and about half that number when sera from mite-allergic children were used. The reason(s) for the lower rate of reactivity observed with sera from children is unknown, but studies suggest that IgE responses to mites in children are directed toward low MW allergens^{29, 30} rather than high MW allergens. It will be important, however, to determine the frequency of reactivity with native amylase with RAST or dot-blot analysis, since reactivity toward some mite allergens, for example *Der p I*, has been demonstrated to be significantly higher when these assays are used.

It has been reported that only 43% of sera from mite-allergic adults recognized *Der p* I when sera were examined by immunoblotting,⁶ a value approximately half the value expected on the basis of previous immunochemical studies.

The allergenicity of amylase was found to be dependent on the integrity of intrachain disulfide bonds since it was diminished on previous reduction with DTT, suggesting that the allergenic epitopes are conformational rather than linear. It is possible that the mite amylase described in this study is similar to an uncharacterized mite allergen previously described⁶ on the basis of both MW and loss of allergenicity on reduction in SDS-PAGE immunoblotting studies. An allergen with an MW of 54,000 has also been reported in extracts of the storage mite, *Lepidoglyphus destructor*.³¹

In conclusion, these data described in this study demonstrate that mites from the genus *Dermatophagoides* produce amylase that is allergenic. This molecule now represents the third mite allergen to reveal enzymatic activity. Given the observations that respirable mite feces contain a variety of digestive enzymes, such as lipase, lysozyme, and glucoamylase,¹³ it is feasible that some of the remaining allergens will be demonstrated to be enzymatically active. In addition, occupational exposure to amylases from other sources, such as *A. oryzae* and porcine pancreatic powder, has been reported to induce the production of enzyme-specific IgE and respiratory symptoms after bronchoprovocation with the enzyme.^{16, 32, 33} These data, together with data from the mite study described in this article, suggest that amylase per se is a relatively potent allergen.

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REFERENCES

1. International Workshop Report. Dust mite allergens and asthma: A worldwide problem. Bull WHO 1988;66:769-80.
2. Lind P, Lowenstein H. Identification of allergens in *Dermatophagoides pteronyssinus* mite body extract radioimmuno-electrophoresis with two rabbit antibody pools. Scand J Immunol 1983;17:263-73.
3. Krilis S, Baldo BA, Basten A. Antigens and allergens from the common house dust mite *Dermatophagoides pteronyssinus*. Part II. Identification of the major IgE-binding antigens by crossed radioimmuno-electrophoresis. J ALLERGY CLIN IMMUNOL 1984;74:142-6.
4. Stewart GA, Butcher A, Lees K, Ackland J. Immunochemical and enzymatic analyses of extracts of the house dust mite *Dermatophagoides pteronyssinus*. J ALLERGY CLIN IMMUNOL 1986;77:14-24.
5. Tovey ER, Baldo BA. Comparison by electroblotting of IgE-binding components in extracts of house dust mite bodies and spent mite culture. J ALLERGY CLIN IMMUNOL 1987;79:93-102.
6. Baldo BA, Ford SA, Tovey ER. Towards a definition of the complete spectrum and rank order of importance of the allergens from the house dust mite, *Dermatophagoides pteronyssinus*. Adv Biosciences 1989;74:13-31.
7. Chua KY, Stewart GA, Thomas WR, Simpson RJ, Dilworth RJ, Plozza TM. Sequence analysis of cDNA coding for a major house dust mite allergen, *Der p* I: homology with cysteine proteases. J Exp Med 1988;167:175-82.
8. Chua KY, Doyle CR, Simpson RJ, Turner KJ, Stewart GA, Thomas WR. Isolation of the major allergen *Der p* II by IgE plaque assay. Int Arch Allergy Appl Immunol 1990;91:118-23.
9. Stewart GA, Thompson PJ, Simpson RJ. Protease antigens from house dust mite [Letter]. Lancet 1989;2:154-5.
10. Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TAE. Antigenic and structural analysis of group II allergens (*Der f* II and *Der p* II) from house dust mites (*Dermatophagoides* spp). J ALLERGY CLIN IMMUNOL 1989;83:1055-67.
11. Tovey ER, Chapman MD, Platts-Mills TAE. Mite faeces are a major source of house dust allergens. Nature 1981;289:592-3.
12. Bowman CE. Comparative enzymology of economically important astigmatid mites. In: Griffiths DA, Bowman CE, eds. Acarology VI. Chichester: Ellis Horwood, 1984;2:993-1001.
13. Stewart GA, Lake FR, Thompson PJ. Faecally derived hydrolytic enzymes from the house dust mite: physicochemical characterisation of potential allergens [submitted]. Int Arch Allergy Appl Immunol.
14. McWilliam AS, Stewart GA. Production of multilamellar, small unilamellar and reverse-phase liposomes containing house dust mite allergens: potential adjuvants in the immunotherapy of allergic disease. J Immunol Methods 1989;121:53-60.
15. Flindt MLH. Allergy to alpha-amylase and papain. Lancet 1979;1:1407-8.
16. Baur X, Weissman KJ, Wuthrich B. Enzymes are the allergenic components of inhaled pancreatin. Dtsch Med Wochenschr 1984;109:257-60.
17. Colloff MJ, Stewart GA, Thompson PJ. House dust acarofauna and *Der p* I equivalent in Australia: the relative importance of *Dermatophagoides pteronyssinus* and *Euroglyphus maynei* [in press]. Clin Exp Allergy.
18. Bowman CE, Childs M. Polysaccharides in Astigmatid mites (Arthropoda: Acari). Comp Biochem Physiol 1982;72B:551-7.
19. Peterson GL. Determination of total protein. Methods Enzymol 1983;91:95-119.
20. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680-5.
21. Tovey ER, Ford SA, Baldo BA. Enhanced immunodetection of blotted house dust mite protein allergens on nitrocellulose following blocking with Tween 20. Electrophoresis 1989;10:243-9.

22. Ward LD, Reid GE, Moritz RL, Simpson RJ. In: Villafranca JJ, ed. Current research in protein chemistry [in press]. Orlando, Fla.: Academic Press, 1990:179-90.
23. Begg GS, Simpson RJ. In: Wittmann-Leibold B, ed. Methods in protein sequence analysis. Berlin: Springer-Verlag, 1989: 108-11.
24. Loyter A, Schramm M. The glycogen-amylase complex as a means of obtaining highly purified alpha-amylases. *Biochim Biophys Acta* 1962;65:200-6.
25. Karn RC, Malacinski GM. The comparative biochemistry, physiology and genetics of animal alpha-amylases. *Adv Comp Physiol Biochem* 1978;7:1-103.
26. Toda H, Kondo K, Narita N. The complete amino acid sequence of Taka amylase. *Proc Jpn Acad* 1982;58B:208-12.
27. Wojtowicz MB, Brockerhoff H. Isolation and some properties of the digestive amylase of the American lobster (*Homarus americanus*). *Comp Biochem Physiol* 1972;42B:295-302.
28. Bowman CE, Lessiter MJ. Amylase and esterase polymorphisms in economically important stored product mites (Acari: Astigmata). *Comp Biochem Physiol* 1985;81B:353-60.
29. Tang RB, Tsai LC, Hung MW, Hwang B, Wu KG. Detection of house dust mite allergens and immunoblot analysis in asthmatic children. *J Asthma* 1988;25:83-8.
30. Stewart GA, Thompson PJ, Doyle C, Signor C. Immunoglobulin responses to house dust mite allergens in mite-allergic asthmatics. *N Engl J Allergy Proc* 1988;9:294.
31. Johansson E, van Hage-Hamsten M, Johansson SG. Demonstration of allergen components in the storage mite *Lepidoglyphus destructor* by an immunoblotting technique. *Int Arch Allergy Appl Immunol* 1988;85:8-13.
32. Weissman KJ, Baur X. Occupational lung disease following long-term inhalation of pancreatic extracts. *Eur J Respir Dis* 1985;66:13-20.
33. Baur X, Fruhman G, Haug B, Rasche B, Reiher W, Weiss W. Role of *Aspergillus* amylase in baker's asthma. *Lancet* 1986;1:43.

Effects of platelet-activating factor on bioelectric properties of cultured tracheal and bronchial epithelia

**Jun Tamaoki, MD, Noritaka Sakai, MD, Kazuo Isono, MD,
Toshinori Kanemura, MD, Isao Yamawaki, MD, and Takao Takizawa, MD**
Tokyo, Japan

To elucidate the effect of platelet-activating factor (PAF) on ion transport function of airway epithelial cells, we studied bioelectric properties of cultured tracheal and bronchial epithelia from dogs under short-circuit conditions in vitro. Addition of PAF (10^{-5} mol/L) to mucosal solution of Ussing chamber increased short-circuit current of tracheal epithelium from 3.3 ± 0.7 to 8.5 ± 1.2 $\mu\text{A}/\text{cm}^2$ ($p < 0.001$). This effect was dose dependent, and there was a corresponding increase in transepithelial potential difference. In contrast, PAF was without effect when it was added to the submucosal side. Electrical properties of bronchial epithelium remained unchanged by PAF. The PAF-induced increase in short-circuit current was not affected by amiloride but abolished by diphenylamine-2-carboxylate, bumetanide, or Cl-free medium. The effects of PAF were not altered by AA-861 or U-60257, but attenuated by indomethacin and piroxicam, and dose-dependently blocked by CV 6209 and WEB 2086. Mucosal, but not submucosal, addition of PAF increased the rate of prostaglandin release from tracheal epithelium. These results suggest that PAF selectively stimulates Cl secretion across tracheal epithelium, probably through activation of its specific receptors and the subsequent production of prostaglandins. (J ALLERGY CLIN IMMUNOL 1991;87:1042-9.)

From the Pulmonary Division, First Department of Medicine, Tokyo Women's Medical College, Tokyo, Japan.

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Reprint requests: Takao Takizawa, MD, First Department of Medicine, Tokyo Women's Medical College, 8-1 Kawada-Cho, Shinjuku, Tokyo 162, Japan.

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