Allergenicity and Physicochemical Characterization of House Dust Mite Derived Amylase

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Abstract

The enzyme amylase was shown to be present in extracts prepared from both house dust and spent growth medium used in the culture of the mite *Dermatophagoides pteronyssinus*. In dust, it was shown to correlate with both mite counts and concentrations of the faecally derived mite allergen, *Der p* I. Mite amylase was isolated from the culture medium and shown to be a single chain protein with a molecular weight of 56,000. The enzyme contained free sulphhydryl groups and had the N-terminal sequence, KYXNPHFIGX-RSVITXLME. It was found to be an allergen using sera from adults (46% positive) and children (25%) who were mite allergic. The expression of allergenicity was dependent on the integrity of intra-chain disulphide bonds.

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Studies from our laboratory have shown two of the important allergens, designated Derp I and III from the house dust mite, *Dermatophagoides pteronyssinus*, are proteolytic enzymes [1,2]. These observations suggested other mite-derived enzymes, particularly those involved in mite digestion, might also be allergenic and particular attention was thus directed to a study of amylase. Amylase activity was assayed using a commercial amylase assay and an agarose-starch plate method [3]. Enzyme activity was easily detected in extracts of whole mite and spent growth medium (which was devoid of whole mites) but not in unused growth medium, suggesting it was present in faecal pellets [4]. Solubility data, which showed that the enzyme eluted from dry medium within 40 s after contact with an aqueous solvent, were consistent with this proposal [5]. Amylase was also found in 20 out of 20 extracts of dust obtained from mattresses (geometric mean 1.95; range 0.6–15.3 units/g fine dust) and in 18 out of 20 from lounge room carpets (geometric mean 1.83; range 0.6–4.4 units/g fine dust). Whilst its derivation from mites was not formally proved, it correlated with both mite counts ($n = 40$; $r = 0.35$, $p < 0.05$) and with Derp I concentrations ($n = 40$; $r = 0.41$, $p < 0.01$).

Mite amylase was isolated from the growth medium by its capacity to bind to shellfish glycogen [4, 6]. Glycogen was added to medium dissolved in 40% (v/v) ethanol at 0°C and the resulting complex, which precipitated under these conditions, was isolated. The glycogen was removed by utilizing the inherent hydrolytic activity of the mite amylase present and the enzyme further purified by gel filtration and chroma-tofocusing. Mite amylase was shown to be similar to amylases from mammalian sources with regard to apparent molecular weight (56,000 by SDS-

PAGE) and the presence of free sulphhydryl groups, as judged by its binding to an organomercurial affinity chromatography matrix. It was heterogeneous with regard to charge, with p_i in the range 5–7. The N-terminal sequence of the enzyme was shown to be KYX-NPHFIGXRSVITXLME and a search of the se-

358

Lake/Ward/Simpson/Thompson/Stewart

Table 1. A summary of the biochemical and physicochemical properties of the major mite allergens

‘ Molecular weight data derived from cDNA studies [1,9],

2 gel filtration and SDS-PAGE studies [5], and

3 SDS-PAGE studies [4]. Enzyme activity data obtained from refs. 2,4, 5.

4 Tentative assignment

quence data banks failed to reveal homology with any known protein [4].

IgE immunoblot studies were performed using sera from mite-allergic individuals. These studies showed the enzyme was allergenic and, in addition, that its expression was dependent on the integrity of intra-chain disulphide bonds [4]. IgE in sera from both mite-allergic children and adults recognized the enzyme with frequencies of 25 and 46% respectively. These data are consistent with the concept that several major mite allergens are hydrolytic enzymes involved in digestion [2, 4, 5], and table 1 summarizes their characteristics to date. Amylases from fungal and mammalian sources [7, 8] have also been shown to be allergenic, raising the possibility that biochemical activity may be an important sensitizing attribute of such molecules.

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