

An IgE-binding TAME esterase in the urine of the mouse, *Mus musculus domesticus*

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1. Summary

Among the highly heterogeneous components of the mouse urinary protein complex (MUP) a hydrolase was detected which was capable of splitting the proteinase ester substrate TAME as well as a synthetic chromogenic tripeptide specific for tissue and urinary kallikreins. The binding of mouse-specific IgE antibodies from the serum of a highly mouse-allergic patient occurred preferentially to this kallikrein-like enzyme. This finding underscores the possible association of significant biological activities with predominant IgE-binding allergens, especially in view of the strongly sensitizing potential and the known messenger functions of the MUP proteins.

2. Introduction

The allergens causing human respiratory allergy to animals are known to be associated with (airborne) emanations or secretions as represented by the (dried) particles of the sweat (skin, dander, fur), saliva and urine. Other potential sources of allergens are the dried and dusty excreta of mammalian and non-mammalian organisms [1]. Hypersensitivity disease provoked by inhalation of such organic materials most often occurs in so-called 'atopic' patients coming into contact with household pets or other domestic animals. Clinical hypersensitivity reactions classified as 'occupational animal allergy' have particularly been observed in research establishments among workers exposed to laboratory animals, especially rodents [2,3]. The respiratory, cutaneous or ocular allergy symptoms have been attributed

to aero-allergens dispersed in the environment and derived from the skin and, more specifically, the urine of these small animals [4]. In the blood serum of affected patients specific IgE and IgG antibodies against the allergenic components of the sensitizing animal source can usually be demonstrated [5]. A predominant sensitizing allergen in urine of the mouse has been identified as a pre-albumin belonging to the family of so-called major urinary proteins (MUP). The molecular size was reported as 16–17 kDa in the unreduced state and 22 kDa in the reduced form, but the broad zone of isoelectric points (pI 4.2–4.9) indicated substantial heterogeneity [6–9]. Indeed, published staining profiles of the MUP complex after isoelectric focusing in polyacrylamide gels disclose a minimum of 20–30 distinct protein components [10]. Since the physicochemical characteristics of the mouse protein allergens are very similar to those reported for the urinary and glandular kallikreins [11], and in view of the known physiological effects of these kininogenases, it seemed worthwhile to pursue the possible relationship in more detail. The present paper reports that the IgE-binding mouse urinary allergens indeed occur in close association with urinary proteinase(s) capable of hydrolysing the synthetic ester substrate *N*-*p*-tosyl-L-arginine methyl ester (TAME).

3. Materials and Methods

Urine of inbred female Swiss mice killed as untreated controls in immunological studies was collected by sterile bladder puncture. A pooled volume of 12 ml of urine was dialysed from Visking dialysis tubing (nominal exclusion limit: 10 kDa) against two changes of distilled water for 24 h at 4°C, and the retentate was lyophilised to produce 62 mg of non-hygroscopic un-

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dialysable protein material, representing a yield of 5.2 g/l. Stratum corneum scraped from the skin of dead mice was suspended in acetone and the allergenic proteins were isolated from the skin flakes as described for human dander allergens [12].

The enzyme activity on the substrate TAME was established by a colorimetric method. Of each eluate fraction off a chromatographic column, 1 ml was mixed with 0.3 ml of 1% TAME (British Drug Houses, Poole, UK) in 0.5 ml of 0.1 M phosphate buffer (pH 7.4), and the mixture was kept at 37°C for 60 min. The enzymatic reaction was terminated by adding 0.5 ml of 15% trichloroacetic acid. The methanol released was then determined colorimetrically by pipetting 1 ml of the reaction mixture into a tube containing 0.1 ml of 2% KMnO_4 , followed by 0.2 ml of Na_2SO_3 and 4 ml of sodium chromotropic acid (Merck, Darmstadt, Germany) in concentrated sulphuric acid. The mixture was heated for 15 min at 100°C, cooled in ice-water and evaluated against a standard curve by reading the optical density in a spectrophotometer at 580 nm.

Enzymatic activity was also determined with the substrate D-valyl-L-leucyl-L-arginine-*p*-nitroanilide-di-HCl (S-2266, Kabi-Pharmacia, Sweden) in plastic tubes containing 200 μl of the pooled chromatographic enzyme fraction at 5 mg/ml, 700 μl of 0.1 M Tris-HCl buffer (pH 8.2) and 100 μl of S-2266 (1.5 μM /ml) for 75 min at 37°C. The reaction was terminated by the addition of 100 μl of 50% acetic acid, and the optical densities were read at 410 nm.

Allergen-coated paper discs for IgE antibody capture from human allergic serum were prepared by coupling 25 mg of total dialysed urinary protein per 100 cellulose discs (5 mm diameter, Whatman no. 1 filter paper) pre-activated with cyanogenbromide by the method of Ceska et al. [13]. After the conjugation reaction, the discs were washed 3 times with ice-cold 0.1 M NaHCO_3 and then incubated in the cold with 50 ml of 0.3 M ethanolamine. After 3 more washing steps with NaHCO_3 , the discs were treated with 0.1 M sodium acetate buffer (pH 4.0) and finally washed exhaustively with water. Binding of specific IgE from the serum of a highly mouse-allergic laboratory research worker was established by RAST inhibition as described by Yman et al. [14].

4. Results

In a representative experiment, a 20 mg dialysed and lyophilised sample of mouse urinary protein was fractionated by gel filtration on a 90 $\times$ 2.5 cm column of Sephadex G-100 suspended in 0.9% NaCl. Material was eluted from the column with the same solvent. Auto-

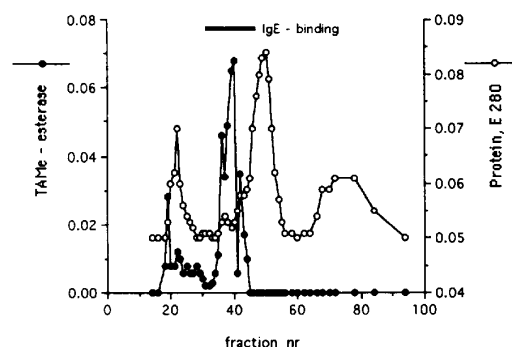


Fig. 1. Gel filtration of 20 mg mouse urine HMW on a 90 $\times$ 2.5 cm chromatographic column of Sephadex G-100 and eluted with 0.9% NaCl. Protein concentration (by UV absorption at 280 nm), TAME-esterase activity and IgE-binding potential (by RAST inhibition) of the eluate fractions are shown. Enzyme-containing fractions 36–42 were pooled, dialysed and used for heat stability and inhibition studies as well as for the hydrolysis of the kallikrein substrate S-2266.

matically collected 6 ml effluent fractions were analysed for TAME esterase activity and pooled according to the enzyme and protein peaks as shown in Fig. 1. Pools were adjusted to equal protein concentration and the IgE-binding potency was evaluated by RAST inhibition, using the serum of a mouse-allergic patient with a high level of mouse-specific IgE antibodies. As shown in Fig. 1, the most pronounced TAME hydrolase enzyme of mouse urine coincided closely with the bulk of the IgE-binding activity but not with the main protein peak.

The pooled enzyme fraction was found to hydrolyse the chromogenic tripeptide substrate S-2266 with known specificity for tissue and urinary kallikreins. Enzyme activity was not abolished by incubation with human serum or the proteinase inhibitor Trasylol. Although heating the enzyme solution for 10 min at 100°C com-

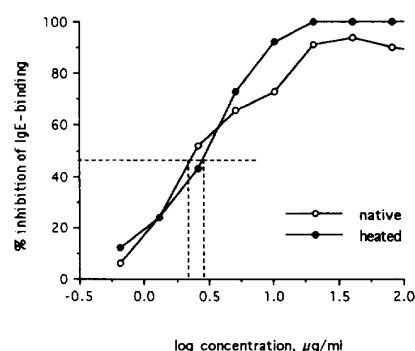


Fig. 2. Dose-response curve for determining the IgE-binding potency (by RAST inhibition) of the native and heated (10 min at 100°C) allergenic proteins in the chromatographic enzyme pool.

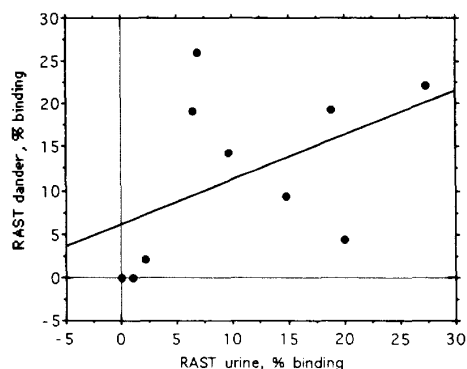


Fig. 3. Relationship between direct RAST values (expressed in % binding from a standard dose of radiolabelled goat anti-IgE) against mouse skin epithelium (acetone-defatted dry dander) and mouse urinary proteins (MUP) in the sera of 10 mouse-allergic patients. Equation of the regression line: $y = 0.51x + 6.21$, $r = 0.48$, $P < 0.1$.

pletely destroyed the catalytic activity, the IgE-binding capacity (i.e., the point of 50% inhibition) remained virtually unaffected (Fig. 2).

It has been pointed out that IgE-binding mouse urinary proteins of pI 4.9 crossreact extensively with the allergens in mouse pelt, hair follicles and dander [6,7,9]. As shown in Fig. 3, comparative IgE analysis by direct RAST with mouse urinary or dander proteins in 10 serum samples of individual mouse-allergic patients indeed showed a significant, though not absolute relationship, confirming our previous results [15].

5. Discussion

The MUP complex is known to be highly heterogeneous as revealed by the multitude of protein bands observed after isoelectric focusing (IEF) in polyacrylamide gel [10]. This finding was confirmed in our study, where IEF of the urinary proteins demonstrated at least 25 closely affiliated bands in the pI 3–5 range (data not shown). The enzymatic analyses in the present investigation confirm that the MUP complex embodies urinary kallikreins capable of hydrolysing the serine proteinase substrate TAME as well as the kallikrein substrate S-2266. The enzyme(s) strongly bind specific anti-mouse IgE from human allergic serum. However, although heat treatment abolishes the enzymatic activity, the antigenic determinants recognized by IgE antibody remain unaffected, indicating that the main epitopes are located on the secondary structure. In our experience the same is true for the allergenic proteinases from the house dust mite *Dermatophagoides pteronyssinus* and from the dander and urine of cats and dogs.

In light of the existing evidence, it seems undesirable to identify the MUP complex as a (single) major mouse allergen designated as *Mus m* I, even though the constituent pre-albumins and proteinases bind specific IgE antibodies [8,16]. Indeed, Twiggs et al. [10] found no relationship between a positive RAST and skin test end-point titration with mouse urinary allergens and considered that the discrepancy merits further investigation. Mouse urinary proteins have been shown to carry significant biological information, e.g., as HLA-linked odour-binding and sex-related proteins [17]. Other ligands include unique chemosignals like terpenic compounds and hormones [18]. It is worth noting in this respect that the mouse androgen-binding proteins have recently been shown to share partial identity with the peptide chain 1 of the predominant cat allergen *Fel d* I and with rabbit uteroglobin [17]. Hence, in reviewing the allergenicity of environmental (occupational) allergen sources it may perhaps be worth the effort to consider possible allergy-related biological actions distinct from antigenic IgE antibody binding.

Under natural conditions, the exposure of patients is not restricted to just the IgE- or IgG-binding antigens from an allergenic substrate. It is conceivable that the kininogenase action of enzymatically active kallikreins upon contact with the mucous membranes may in non-specific fashion trigger the specific immunological response to bystander antigens and to the enzyme molecule itself. Mouse glandular kallikreins have indeed been found to enhance spontaneous lymphocyte proliferation [19]. In this sense, the finding of proteinases as IgE-binding allergens in mouse urine may be another example of sensitizing agents with a dual action, i.e., as adjuvants and immunogens [20].

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