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ORIGINAL ARTICLE

Non-protease native allergens partially purified from bodies of eight domestic mites using p-aminobenzamidine ligand

T. Erban[a,*], R. Klubal[b]

[a] *Crop Research Institute, 507/73, Prague 6-Ruzyne, CZ-16106, Czech Republic*
[b] *Medical Center Prague, Mezi Vodami 205/29, Prague 4-Modrany, CZ-14300, Czech Republic*

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Abstract
Background: Optimised purification steps for concentrating trace target native antigens are needed. Combining the p-aminobenzamidine ligand with protease inactivation enables partial purification of mite non-protease allergens lacking proteases.
Objective: We sought to analyse in detail proteins obtained using this method from eight species of synanthropic acaridid mites and tested IgE reactivity using pooled human sera.
Materials and methods: Proteins affinity bound to p-aminobenzamidine as a ligand were identified by MALDI TOF/TOF. After electroblotting, the proteins were visualised using the fluorescent SYPRO-Ruby protein blot stain, and IgE reactivity was further analysed using pooled human sera collected from patients allergic to house dust mites.
Results: MS/MS identification confirmed previous results that no proteases were purified. Protein patterns corresponding to the allergens Der f 7, Der f 30 and actins indicated that these proteins are purified using p-aminobenzamidine and are present across a wide spectrum of acaridid mites. When using *Dermatophagoides farinae*, apolipophorins (Der f 14), chitinase-like Der f 15 and 18, 70-kDa heat shock protein, and a Der f Alt a10 allergen homolog (gi|37958173) were also detected. The target antigens tropomyosins and paramyosins showed similar IgE binding among the mite species tested. IgE reactivity with miscellaneous *D. farinae* antigen was also observed.
Conclusions: Partial purification of mite non-protease antigens using a strategy combining p-aminobenzamidine with protease inactivation was verified by 1D-E and 2D-E analyses. IgE binding to p-aminobenzamidine-purified native non-protease mite antigens was tested using pooled sera. This preliminary study allows for further work on individual serum samples, allowing confirmation of immunoreactivity.

* Corresponding author.
E-mail address: arachnid@centrum.cz (T. Erban).

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Introduction

Compared to native forms of proteins, differences in biological activity can be observed with recombinant proteins.[1–3] For example, variations in immunoreactivity have been found between native and recombinant antigens such as mite tropomyosin[4] and paramyosin.[5] Thus, there is a need to develop processes for preparation of native antigens, as only native forms of antigens can reveal the true immune response.

Synanthropic acaridid (astigmatid) mite species are typically classified into two artificial groups: house dust mites (HDMs) and stored-product mites (SPMs).[6,7] Both groups are also called ''domestic mites'' because many SPMs of the families Acaridae, Glycyphagidae, and Chortoglyphydae are also found in house dust.[8] Various mites have cross-reactive and species-specific antigens.[9,10] Moreover, cross-reactivity with allergens from other invertebrates, including insects, molluscs, or crustaceans, has been observed for mites.[10–12]

Among the main groups (Grps) of mite allergens, tropomyosins (Grp 10) are considered pan-allergens because they are highly conserved and because these proteins of many invertebrates, including molluscs, parasitic worms and various orders of arthropods, exhibit cross-reactivity. In addition, tropomyosin from some invertebrates is considered a food allergen.[11–17] To date, tropomyosin allergenicity has been identified for 28 invertebrate species (for detailed information, visit http://www.allergen.org), and the tropomyosins from six astigmatid (syn. acaridid) mites, *Blomia tropicalis*, *Dermatophagoides farinae*, *D. pteronyssinus*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae* and *Chortoglyphus arcuatus*, have been identified as allergens (http://www.allergen.org).[4,15,18–20] In contrast to knowledge about tropomyosin, there is limited information regarding allergenicity related to paramyosin. Although only four allergenic paramyosins have been identified, three are of acaridid mite origin, i.e., *B. tropicalis*, *D. farinae* and *D. pteronyssinus*.[21–23] In addition, some invertebrate muscle proteins are allergens, including troponin C from cockroaches (*Blattella germanica*),[24] *Periplaneta americana*, the stored-product mite *T. putrescentiae* and some other invertebrates (*Homarus americanus*, *Crangon crangon* and *Penaeus monodon*) for which these proteins are considered to be food allergens. Given that myosin light chains of *B. germanica*, several species of decapods, and *D. farinae* have been identified as allergens (http://www.allergen.org), it can be speculated that the entire muscle complex of certain invertebrates is allergenic. This assumption supports the recognition of α-actinin involved in muscle contraction and β-actin as new shrimp allergens.[11]

It has been reported that as a ligand, p-aminobenzamidine binds with high affinity to tropomyosins, paramyosins and actins from domestic mites.[25] Binding by p-aminobenzamidine to these proteins has also been confirmed for *B. germanica* and *Oryctolagus cuniculus*. In this case, the methodology used employed a strategy for inactivating proteases that were not of interest using protease inhibitors during the purification process; thus, proteases were not purified, and only non-protease proteins were obtained.[25] Additionally, the study showed that mite paramyosin is monomeric and dimeric and tropomyosin monomeric and tetrameric using non-reducing sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).[25] The applicability of this method using p-aminobenzamidine to concentrate trace target proteins of *V. destructor* has also been demonstrated.[26]

In this study, we show that the p-aminobenzamidine ligand combined with protease inactivation using inhibitors can concentrate mite non-protease proteins to enable analysis of native antigen immunoreactivity.

Materials and methods

Mites

Eight species of synanthropic acaridid mites, Acari: Acaridida (alternatively Astigmata), were selected for this study because of their medical and economic importance. The mites were cultivated on an IWAKI 25-cm^2 surface area in 70-mL-capacity tissue culture flasks (IWAKI flasks; Cat No. 3100-025; Sterilin, Newport, UK).[27] Two different rearing diets were used to cultivate the wide collection of species. A wheat-derived rearing diet (*Acarus siro*, *Tyrophagus putrescentiae*, *Aleuroglyphus ovatus*) consisted of a mixture of oat flakes, wheat germ and dried yeast extract (Rapeto, Bezdruzice, Czechia) (10:10:1 w/w). A fish food-derived rearing diet (*Tyroborus lini*, *Blomia tropicalis*, *Dermatophagoides farinae*, *Glycyphagus domesticus*, *Lepidoglyphus destructor*) was composed of dog food (Ontario-pet, Placek, Podebrady, Czechia), wheat germ, dried fish food (LonBio, AquaTropic Lonsky, Praha, Czechia), Pangamin and gelatin (Serva Electrophoresis, Heidelberg, Germany) (10:10:3:2:1 w/w). All the biological samples originated from the rearing facility at Crop Research Institute (http://www.vurv.cz), Prague, Czechia. Mites were reared in small amounts of diet analogously to described.[28] The mites (minimal purity: 95% pure mites) were separated from the spent growth medium, allowed to defecate for 24 h, and then collected.

Protein extraction using protease-blocking extraction buffer

A 0.1 g sample of pure mite bodies was homogenised in a sterilised glass Potter-Elvehjem homogeniser (Art. No. 6305; Kartell Labware division, Noviglio, Italy) in 1 mL cold 0.01 M phosphate-buffered saline with 1% CHAPS (w/w) and 20 μL protease inhibitor mixture (Cat No. 80-6501-23, GE Healthcare Life Sciences, Uppsala, Sweden). Samples were homogenised three times using a drilling machine for 2 min each, followed by 20 min of cooling on ice. Next, 0.5 mL extraction buffer was added, and homogenisation was repeated three times for 1 min each. The homogenate was allowed to stand for 10 min on ice, and the supernatant was transferred to a centrifuge tube (Orange Scientific, Braine-l'Alleud, Belgium) and centrifuged for 15 min at 10,000 × *g* and 4 °C using an MR 23i centrifuge (Jouan Industries, France). The supernatant was filtered using a glass Luer-lock syringe and 0.45-mm regenerated cellulose filter (TR-200435, Teknokroma, Barcelona, Spain).

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