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Lack of Lysozyme Activity of Natural and Yeast-Derived Recombinant Der p 2

Key Words

Yeast, baker
House dust mite
Lysozyme
Natural Der p 2
Recombinant Der p 2
Saccharomyces cerevisiae

Abstract

For *Dermatophagoides pteronyssinus* a number of allergens have been reported. Although the function of several allergens is known, that of the house dust mite allergen Der p 2 is unknown. A role as lysozyme has been suggested. We tested a yeast-derived recombinant Der p 2 for its lysozymatic activity. This recombinant allergen and affinity-purified natural Der p 2 lacked this enzymatic activity. Whole-body mite extract retained its lysozymatic activity after removal of the group 2 allergen by monoclonal antibody depletion. Analysis of spent medium revealed the reciprocal: it contained group 2 allergen, but lacked lysozymatic activity. Together, these data demonstrate that mite lysozyme and Der p 2 are different components of mite extract.

Introduction

For the house dust mite *Dermatophagoides pteronyssinus* (or *D. farinae*) 12 allergens have been reported so far [1]. Much effort has been made to elucidate the function of these house dust mite allergens. The function of the major allergen Der p 2, however, remains unknown. The cDNA coding the group 2 allergen has been isolated by Chua et al. [2]. A role of Der p 2 as lysozyme has been suggested [3–5], but has so far not been confirmed by experimental data. A number of similarities between both proteins, such as acid and heat stability and molecular weight, have been reported [4]. Furthermore, Der p 2 and lysozyme are both present in mite body and fecal extracts [6]. Recently, homology of Der p 2 with a mammalian epididymous protein has been reported [7]. In this study, recombinant Der p 2, expressed in yeast, and affinity-purified natural Der p 2 were used to investigate whether Der p 2 has lysozyme activity.

Material and Methods

Monoclonal Antibodies

The anti-Der p 2 monoclonal antibodies (mAbs) used were α DpX [8] and 10E11 [Hakkaart et al., manuscript submitted].

Lysozyme Assay

A *Micrococcus lysodeikticus* (Millipore, Bedford, Mass., USA) solution was prepared as follows: 0.2 mg/ml bacterial suspension were dissolved in 66.7 mM Na₂HPO₄/NaH₂HPO₄ buffer, pH = 6.2. 2,500 μ l of this bacterial suspension were mixed with 500 μ l of the sample. The turbidity at 540 nm was determined spectrophotometrically during 20 min (every 30 s). As a positive control, hen egg white lysozyme (HEL; Pharmacia Biotech, Uppsala, Sweden) was used.

Der p 2 Sources

Mite Der p 2. Natural Der p 2 was affinity purified by means of mAb α DpX, as described previously [7]. The same affinity matrix was used for depletion of mite extract. Depletion efficiency was checked by Der p 2 ELISA (>99%).

Yeast-Derived Der p 2. The production of recombinant Der p 2 expressed in yeast and its purification by means of gel filtration is described elsewhere [Hakkaart et al., manuscript submitted].

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Measurement of Mite and Recombinant Der p 2

Recombinant and natural Der p 2 were measured in a two-site ELISA as described [Hakkaart et al., manuscript submitted] with mAb 10E11 as catching antibody and mAb α DpX as detecting antibody. Purified natural and recombinant Der p 2 with known protein content were used as standards.

Results

Der p 2 was purified from mite extract and yeast growth medium. The purified allergen molecules (>95% purity as judged by SDS-PAGE/silverstaining) were tested for their lysozyme activity, together with whole mite extract (fig. 1). Mite extract demonstrated lysozymatic activity while purified Der p 2 – either recombinant or natural – lacked this activity. The influence of pH fluctuation during affinity chromatography was studied by incubation of mite extract in glycine, pH = 2.5, for 1 h, after which $\frac{1}{2}$ volume of 1.0 M Tris, pH = 9, was added. This treatment did not show an effect on the lysozymatic power of the whole-body extract (results not shown). Der-p-2-depleted body extract was studied as well. The Der p 2 concentration and protein content were measured. The whole-body extract contained 30 μ g/ml Der p 2 and 1,300 μ g/ml protein, while the depleted extract contained 1.4 ng/ml and 950 μ g/ml, respectively, indicating that the extract was diluted 1.4 times during depletion. The depleted and non-depleted mite extracts were compared with respect to their lysozymatic activity at equal protein concentrations. Removal of >99% Der p 2 from the body extract did not have any influence on the enzymatic activity of the solution.

Spent medium – in contrast to whole-body extract – lacked lysozymatic activity but contained 2 μ g/ml Der p 2 (results not shown).

Discussion

The mature allergen molecule of Der p 2 expressed in the baker's yeast *Saccharomyces cerevisiae* shows a good immunological resemblance to mite-derived Der p 2 [Hakkaart et al., manuscript submitted]. Here, we demonstrated that this Der p 2 lacked lysozymatic activity. Since no denaturing steps were carried out during the isolation of the recombinant protein, we anticipate that if any potential enzymatic activity was present, it should have been retained. Furthermore, different kinds of lysozymes expressed in *S. cerevisiae* were shown to be enzymatically active [9, 10]. Nevertheless, it cannot be completely ruled out that the (supposed) lysozymatic character of Der p 2 was not re-

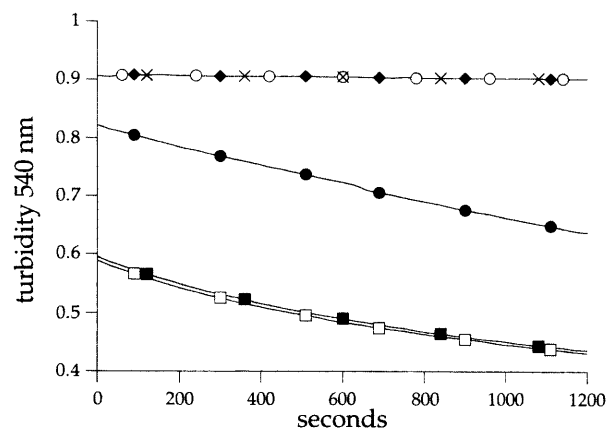


Fig. 1. Lysozymatic activity of Der p 2 and (depleted) mite extract. Mite extract and purified Der p 2 preparations were diluted in phosphate buffer to a Der p 2 concentration of 2 μ g/ml. The depleted mite extract was adjusted to an equal protein content as the body extract. The turbidity of the bacterial suspension at 540 nm, during 20 min is presented. X = Phosphate buffer; O = yeast Der p 2; $\blacklozenge$ = mite Der p 2; $\bullet$ = HEL; $\blacksquare$ = mite extract; $\square$ = depleted mite extract.

tained in its recombinant counterpart. Therefore, Der p 2 was also purified from whole body mite extract. This natural Der p 2 preparation lacked lysozymatic activity as well. Next we studied the lysozymatic activity of the depleted whole-body extract. Removal of the group 2 allergen from this extract had no influence on its capacity to degrade the bacterial suspension.

Together, these findings demonstrate that the lysozymatic activity of the house dust mite cannot be ascribed to the group 2 allergen, but rather to another mite protein.

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Announcement

10th Gold Medal of the FARE 1998

The next Gold Medal of the Foundation for Allergy Research in Europe (FARE) will be awarded during the 17th European Congress of Allergology and Clinical Immunology (EAACI) in Birmingham, June 1998.

The winners of the Gold Medal in the past were: Prof. S. G. O. Johansson, Stockholm/S (1971); Dr. B. Weeke, Copenhagen/DK (1974); Prof. J. Bach, Paris/F (1977); Dr. A. Loewenstein, Copenhagen/DK (1980); Dr. W. Parish, Bedford/UK (1983); Dr. C. André, Lyon/F (1986); Prof. D. Vervloet, Marseille/F (1989); Dr. R. Valenta, Vienna/A (1992), and Prof. G. Pauli, Strasbourg/F (1995).

The topic chosen by the FARE for the Gold Medal 1998 is: 'Measures to prevent atopic diseases in early childhood.'

The participation is open to scientists working in Europe independent of their nationality or their type of work (clinic, institute, private practice).

Unpublished or recently published papers with an extended summary of the results about the chosen topic may be submitted, provided that they have not been submitted elsewhere for a prize or similar award.

The applications in 14 copies, together with a curriculum vitae and a list of publications should be in the hands of the secretary/treasurer of the FARE not later than January 10, 1998. The council of the foundation will choose the prize winner from the candidates. According to article 5 of the statutes, the Gold Medal of the foundation is a distinction for a young scientist, who has recently contributed important work in the field of allergology. The prize should stimulate the continuation of such research. The Gold Medal 1998 in Birmingham is accompanied by a travel award, if the candidate is not already a speaker or chairman of the EAACI congress.

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