

Biochemical and immunological characterization of a recombinant precursor form of the house dust mite allergen Der p 1 produced by *Drosophila* cells

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Summary

Background The major house dust mite allergen Der p 1 elicits strong IgE antibody responses in patients suffering from mite allergy.

Objective This study reports the expression and characterization of a recombinant precursor form of Der p 1 secreted as ProDer p 1 from insect cells.

Methods The cDNA coding for ProDer p 1 was cloned downstream to the gp67 signal peptide, starting from commercial cDNA encoding Der p 1 and PCR-amplified ProDer p 1 genomic fragment. ProDer p 1, expressed in *Drosophila* cells and purified from culture medium, was compared to Der p 1 isolated from mite culture, in terms of glycosylation, enzymatic activity as well as IgG- and IgE-binding capacity.

Results Sequence analysis of the genomic clone of ProDer p 1 revealed that, besides two introns in the mature Der p 1 coding sequence, two introns were also present in the propeptide coding sequence. ProDer p 1 was purified to homogeneity by a combination of ion-exchange, hydroxyapatite and gel filtration chromatographies. The precursor form of Der p 1 could be processed *in vitro* into mature Der p 1 under acidic and reducing conditions. Carbohydrate analysis clearly indicated that ProDer p 1 expressed from insect cells was glycosylated and that glycan structures were located only in the prosequence. ProDer p 1 displayed a similar immunoreactivity towards IgE, monoclonal and polyclonal IgG antibodies compared to natural Der p 1. Specific activity measurements using synthetic substrates clearly indicated that, contrary to natural Der p 1, ProDer p 1 was totally enzymatically inactive.

Conclusions The expression of an enzymatically inactive and highly antigenic ProDer p 1 zymogen molecule could be a suitable strategy for the development of *in vitro* diagnosis test as well as for specific immunotherapy.

Keywords: IgE, maturation, mite allergy, ProDer p 1

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Introduction

Sensitization to inhalant allergens of the house dust mite *Dermatophagoides pteronyssinus* is commonly associated with allergic diseases such as atopic dermatitis, perennial

rhinitis and bronchial asthma [1]. Amongst these allergens, Der p 1 is considered to be major, which binds IgE in 70–80% of allergic sera [2,3]. Der p 1 is a 30-kDa protein located in the mite gut, suggesting a digestive function and is, indeed, found in high concentration in faecal pellets, which can become easily airborne and inhaled [4,5]. The cDNA coding for Der p 1 has been cloned and sequenced [4,6,7]. This allergen is a protein of 222 amino acid residues

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synthesized as a precursor of 320 amino acid residues, including a 18-amino acid signal peptide and 80-amino acid N-terminal prosequence. The Der p 1 primary structure displayed homologies with that of papain, the cysteine proteinase archetype, including the conservations of the active site residues. The Der p 1 structure, modelled on the crystal structure of papain, shared essential structural and mechanistic features with other papain-like cysteine proteinases [8]. The maturation of ProDer p 1 is not known to date but it is thought that the enzyme is activated by proteolytic removal of the pro-region or via autocatalytic processing.

The potent allergenicity of Der p 1 appears to be related to its proteolytic activity, which has been shown *in vitro* to compromise functions of the innate and adaptive immune systems. Der p 1 increases the permeability of bronchial mucosa, notably by degrading α -antitrypsin, and consequently facilitates access to antigen-presenting cells [9,10]. Cleavage of CD23, the low affinity IgE receptor, by Der p 1, increases IgE stimulation whereas digestion of CD25, the IL-2 receptor, results in a bias toward a Th2 immune response [11,12].

It is generally accepted that recombinant production of allergens is a straightforward strategy to obtain defined materials with high yields for diagnosis and treatment of IgE-mediated allergic disorders [13]. Previous attempts of Der p 1 expression in bacteria and yeast indicated that the allergen was poorly expressed mainly under an insoluble form [14–16]. The present paper describes the cloning and expression of a secreted form of ProDer p 1 produced in insect cells. The purified recombinant allergen was compared to Der p 1 isolated from whole mite cultures, in terms of biochemical, enzymatical and immunological properties.

Materials and methods

Mite cultures

Whole mite culture of *Dermatophagoides pteronyssinus* was provided by Dr Griselet (Université Catholique de Louvain, Belgium). The noncloned European strain of *Dermatophagoides pteronyssinus* was cultured on a 1:1 mixture of TetraMin (TetraMin, Tetra/Second nature, Blacksburg, VA, USA) and dried yeast product according to the method of Miyamoto [17].

PCR amplification of genomic ProDer p 1 DNA

ProDer p 1-encoding DNA was amplified from genomic *Dermatophagoides pteronyssinus* DNA, previously extracted from a whole mite culture, by PCR using the following primers derived from the published complete Der p 1 nucleotide sequence [4,6,7, GenBank accession number U11695 S66377 Y00641]:

5'GCTCTAGACCATGAAAATTGTTTTGGCCATCGCC3' (forward, *Xba*I site underlined) and 5'CGGGATCCCATT-AGAGAATGACAACATATGG3' (reverse; *Bam*HI site underlined). The resulting 1251 bp fragment, proved to be ProDer p 1 genomic DNA after control restrictions, was subcloned in pUC19 after digestion with *Xba*I and *Bam*HI to give pNIV4820.

Construction of the *Drosophila* ProDer p 1 expression vector

An intermediary construction previously designed for baculovirus expression, pNIV4828, was constructed in the pAcGP67A vector (PharMingen, San Diego, CA, USA) containing the gp67 signal sequence. The ProDer p 1 coding cassette was created by the ligation of three fragments: (a) a synthetic fragment coding for a part of the Der p1 propeptide up to the *Eco*RI site located at the end of the sequence. 5'GATCCCCGGCCGT-CATCGATCAAAACTTTTGAAGAAT ACAAAAAAGC-CTTCAACAAAAGTTATGCTACCTTCGAAGATGAA-GAAGCTGCCCCGTA AAAACTTTTTTGAATCAGTAAA-ATATGTTCAATCAAATGGAGGTGCCATCAACCATT TGTCCGATTTGTCGTTGGATG3' (forward) and 5'AAT-TCATCCAACG ACAAATCGGACAAATGGTTGATGG-CACCTCCATTTGATTGAACATAT TTTACTGATTCCAAAAAGTTTTTACGGGCAGCTTCTTCATCTTCGAA-GGTAGCATAACTTTTTGTTGAAGGCTTTTTTGTATTC-TTCAAAAGTTTTGATCGATGACGGCCGGG3' (reverse); (b) an *Eco*RI-*Bss*SI genomic fragment (without introns) from pNIV4820 described hereover; (c) a *Bss*SI-*Pst*I fragment coming from a cDNA encoding mature Der p 1 purchased from ATCC (ATCC clone 87307). The ProDer p1 sequence was cloned downstream to the gp67 signal sequence by insertion in the pAcGP67A expression vector cleaved with *Bam*HI and *Pst*I to give pNIV4828.

A *Spe*I-*Bgl*II fragment from pNIV4828 was cloned in pDS47, a *Drosophila* constitutive expression vector (Invitrogen, Carlsbad, CA, USA) downstream to an *Asp*718-*Spe*I synthetic fragment restoring the gp67 initiation codon: 5'GTACCCCTT-AAGATGCTA3' (forward) and 5'CTAGTAGCATCTTA-AGG3' (reverse). The resulting construction was called pNIV4832.

The final step resulted in the insertion of an *Asp*718-*Not*I fragment from pNIV4832 in the inducible *Drosophila* expression vector pMT/V5-His (Invitrogen) cut with the same restriction enzymes to give pNIV4840.

Expression of the recombinant allergen in drosophila cells

S2 Schneider cells were transfected with pNIV4840 plasmid by calcium phosphate coprecipitation. After a 3-week hygromycin selection (300 μ g/mL), hygromycin-resistant S2 cell population was cultivated in cell factories in DES medium (Invitrogen) supplemented with 10% fetal calf

serum (Gibco Life Technologies, Rockville, MD, USA). For the expression of ProDer p 1, the medium was changed to *Drosophila* serum-free medium (Gibco) and expression was induced by addition of 600 μM CuSO_4 in the medium. Induced cell supernatant was harvested after 3 days and engaged for the purification.

Purification of Der p 1 from natural mite whole body extracts

Whole mite culture was stirred overnight at 4 °C in PBS pH 7.5. Debris and insoluble material were removed by ultracentrifugation at 130000 *g* for 1 h. Solid $(\text{NH}_4)_2\text{SO}_4$ was added to the supernatant to 60% saturation. The precipitate was collected by ultracentrifugation and re-suspended in PBS containing $(\text{NH}_4)_2\text{SO}_4$ 1 M. Proteins were loaded onto a Resource Phenyl column (1 mL, Pharmacia, Uppsala, Sweden) equilibrated in PBS containing $(\text{NH}_4)_2\text{SO}_4$ 1 M. After washing the column with the same buffer, the matrix was washed with PBS containing 400 mM $(\text{NH}_4)_2\text{SO}_4$. Der p 1 was eluted from the column with water. Enriched Der p 1 fractions were pooled, the pH was adjusted to 9 and the conductivity to that of 20 mM Tris-HCl pH 9. The sample was then applied onto a Q sepharose fast flow column (2.6 $\times$ 3 cm, Pharmacia) equilibrated in 20 mM Tris-HCl pH 9. After washing the column with the same buffer, Der p 1 was eluted by addition of 200 mM NaCl in the starting buffer. The Der p 1-containing fractions were pooled and concentrated by ultrafiltration onto a Filtron membrane (Omega serie, cut-off: 10 kDa). The Der p 1 purification was achieved by a gel filtration chromatography onto a superdex-75 column (1 $\times$ 30 cm, Pharmacia) equilibrated in PBS pH 7.3. Purified Der p 1 was concentrated and stored at -20 °C.

Purification of ProDer p 1 from drosophila culture medium

Proteins from *Drosophila* culture medium were concentrated after addition of solid $(\text{NH}_4)_2\text{SO}_4$ to 60% saturation. The pellet was resuspended in 20 mM Tris-HCl, pH 9, and dialysed against the same buffer. After centrifugation at 27200 *g* for 20 min, the supernatant was loaded onto a Q sepharose fast flow column (2.6 $\times$ 3 cm, Pharmacia) equilibrated in 20 mM Tris-HCl pH 9. After washing the column with the same buffer, ProDer p 1 was eluted by addition of 200 mM NaCl in the starting buffer. The ProDer p 1-containing fractions were pooled, the pH was adjusted to 7, and applied onto an hydroxyapatite type I column (1.6 $\times$ 5 cm, Bio-Rad, Hercules, CA, USA) equilibrated in 5 mM $\text{K}_2\text{HPO}_4/\text{K}_2\text{HPO}_4$ pH 7.0. In these conditions, the recombinant allergen went through the resin. The flow-through was concentrated by ultrafiltration onto a Filtron membrane (Omega serie, cut-off: 10 kDa). The ProDer p 1 purification was achieved by a gel filtration chromatography

onto a superdex-75 column (1 $\times$ 30 cm, Pharmacia) equilibrated in PBS pH 7.3. Fractions containing ProDer p 1 were concentrated and stored at -20 °C.

Glycan analysis

Carbohydrate analysis was carried out with the Glycan Differentiation Kit (Boehringer, Mannheim, Germany) using the following lectins: *Galanthus nivalis* agglutinin (GNA), *Sambucus nigra* agglutinin (SNA), *Maackia amurensis* agglutinin (MAA), peanut agglutinin (PNA) and *Datura stramonium* agglutinin (DSA).

Activation of ProDer p 1

Purified ProDer p 1 (100 μg) was applied onto a Sephadex G-25 column (1 $\times$ 15 cm, Pharmacia) equilibrated in 50 mM sodium acetate pH 4 to exchange buffer. Cysteine was added to 20 mM and the sample incubated at 60 °C. At different periods of time, aliquots were neutralized with 1/10 volume of 1 M Tris-HCl pH 9.5. The maturation was monitored by SDS-PAGE.

Enzymatic assays

Enzymatic assays were performed in 50 mM Tris-HCl pH 7, containing 1 mM EDTA and 20 mM L-cysteine at 25 °C in a total volume of 1 mL. Hydrolysis of Cbz-Phe-Arg-7-amino-4-methylcoumarin (Cbz-Phe-Arg-AMC) and Boc-Gln-Ala-Arg-7-amino-4-methylcoumarin (Boc-Gln-Ala-Arg-AMC) (Sigma St Louis, MO, USA) (both substrates at a final concentration of 100 μM) was monitored using a SLM 8000 spectrofluorimeter with λ_{ex} = 380 nm and λ_{em} = 460 nm. Assays were started by addition of cysteine-activated allergen to a final concentration of 100 nM. Before any assay, purified Der p 1 or ProDer p 1 was incubated with a mixture of aprotinin- and p-amino-benzamidine-agarose resins (Sigma) to remove any trace of serine protease activity.

Protein determination

Total protein concentration was determined by the bi-cinchoninic acid procedure (MicroBCA, Pierce) with bovine serum albumin (BSA) as standard.

SDS-PAGE and Western blot analysis

Proteins were analysed by SDS-PAGE on 12.5% polyacrylamide gels. After electrophoresis, proteins were transferred onto nitrocellulose membranes using a semidry transblot system (Bio-Rad). Membranes were saturated for 30 min with 0.5% Instagel in TBS-Tween (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Tween 80) and incubated with

Der p 1 or ProDer p 1 was detected with an ELISA kit using Der p 1-specific monoclonal antibodies 5H8 and 4C1 (Indoor Biotechnologies, Charlottesville, VA, USA).

Immunoplates were coated with Der p 1 or ProDer p 1 (500 ng/well) for 16 h at 4 °C. Plates were then washed five times with 100 μ L per well of TBS–Tween buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.1% Tween 80) and saturated for 1 h at 37 °C with 150 μ L of the same buffer supplemented with 1% BSA (Sigma). Serial dilutions of sera to be tested (sera from allergic and nonallergic patients to *Dermatophagoides*, a kind gift from Prof. Duchateau, Hopital Brugmann, Bruxelles, Belgium) were then incubated for 1 h at 37 °C. Plates were washed 5 times with TBS–Tween buffer and the allergen–IgE complexes were detected after incubation with a mouse antihuman IgE antibody (Southern Biotechnology Associates, Birmingham, AL, USA) and a goat antimouse IgG antibody coupled to alkaline phosphatase (dilution 1/7500 in TBS–Tween buffer). The enzymatic activity was measured using the p-nitrophenylphosphate substrate (Sigma) dissolved in diethanolamine buffer (pH 9.8). Absorbance at 415 nm was measured in a Bio-Rad Novapath ELISA reader.

Total genomic DNA from *Dermatophagoides pteronyssinus* whole culture was used as template for PCR amplification of ProDer p 1 gene. Two fragments of 950 and 1250 bp, respectively, were amplified. The longer one corresponded to the ProDer p 1 gene on the basis of restriction fragment analysis. Sequence determination of the amplified fragment revealed that, besides introns of 65 and 61 bp already located in the mature Der p 1 coding sequence at position 591 and 755 (the nucleotide number starts at first ATG) [19], two small introns of 83 and 60 bp were discovered in the propeptide coding sequence at position 90 and 210, respectively (Fig. 1). Because of the presence of these four introns, the coding cassette for ProDer p 1 was created starting from cDNA encoding Der p 1, PCR amplified ProDer p 1 genomic fragment and oligonucleotide pairs for the junctions. The sequence of the ProDer p 1 clones presented only one difference with respect to previously reported data which led to an amino acid substitution at position 665 (GCA instead of GTA; Val₆₆₅ → Ala) [4,6,7].

The expression of ProDer p 1 was first attempted using the baculovirus expression system in which the ProDer p 1-coding

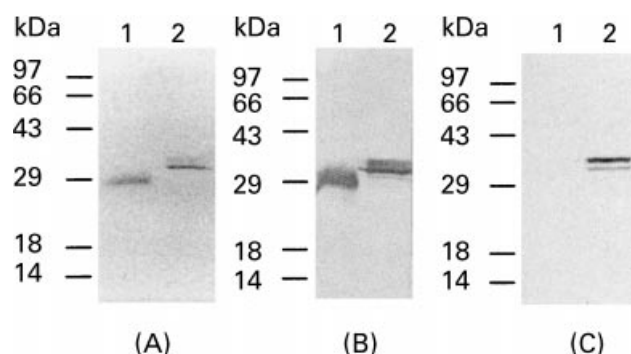


Fig. 2. Purification of ProDer p 1 and Der p 1. Purified allergens were analysed by SDS-PAGE and proteins were detected by Coomassie blue staining (panel A), by immunoblotting (panel B) with rabbit polyclonal serum raised against Der p 1 peptide 245–267 or by lectin staining (panel C) with *Galanthus nivalis* agglutinin (GNA). Lane 1: purified Der p 1. Lane 2: purified ProDer p 1

cassette was inserted in the expression vector pAcGP67A. After selection and subcloning, ProDer p 1 was expressed under a secreted form at high level (more than 20 mg/L culture medium in 24 wells) but the level of production decreased drastically when expression was performed in a larger scale (data not shown). We next focused on the *Drosophila* expression system where we first attempted to produce ProDer p 1 constitutively with the expression vector pDS47/V5-His. No expression could be detected in ELISA or Western blotting (data not shown). In contrast, the immature allergen was efficiently expressed by S2 cells

using the inducible *Drosophila* expression system. The plasmid pNIV4840, carrying the ProDer p 1 coding cassette was introduced in the inducible expression vector pMT/V5-His, then cotransfected with the pCoHygro plasmid into *Drosophila* cells. After a three-week hygromycin selection, the resistant cell population was used for large-scale ProDer p 1 production in cell factories. Addition of 600 μ M CuSO₄ in the serum-free medium led to the expression of ProDer p 1 under a secreted form. The average concentration of ProDer p 1 in spent culture medium was estimated, in a Der p 1 ELISA, at 3 ± 1 mg/L.

Purification and glycan analysis of ProDer p 1

The ProDer p 1 purification was achieved by a combination of anion-exchange, hydroxyapatite and gel filtration chromatographies. The final purification yield was about 1–1.5 mg/L culture medium. After elution from the superdex column, pooled fractions were analysed on SDS-PAGE and by Western blot, identifying three immunoreactive molecules of 34, 37 and 39 kDa, respectively (Fig. 2). This result suggests that the propeptide removal, to yield mature Der p 1, did not occur during the expression and purification steps, as natural Der p 1 migrated on SDS-PAGE as a 29-kDa band. The purity of the product was higher than 90%. Carbohydrate analysis of Der p 1 and ProDer p 1 was performed by glycan recognition with several specific lectins (Fig. 2). ProDer p 1 only reacted with GNA among the five lectins used, pointing to the presence of terminal mannose residues on all immunoreactive species, either as high-mannose N-glycan chains or as exposed mannose in hybrid chains. The lack of reaction with any lectin for natural Der p 1 suggested that this molecule is not glycosylated.

Processing of ProDer p 1

To determine whether ProDer p 1 could be autocatalytically processed into mature Der p 1, purified immature allergen was incubated in acidic buffer in the presence of 20 mM cysteine at 60 °C for different periods of time. Electrophoretic analysis of the processed material revealed that, after an incubation period of 2 h, more than 80% of ProDer p 1 was converted into mature Der p 1 (Fig. 3). Longer exposure did not increase the maturation yield. The requirement for reducing agents appeared essential for the activation process, suggesting that the maturation process depends on a cysteine protease activity.

ProDer p 1 enzymatic activity

The enzymatic activity of ProDer p 1 was determined using Cbz-Phe-Arg-AMC and Boc-Gln-Ala-Arg-AMC, two

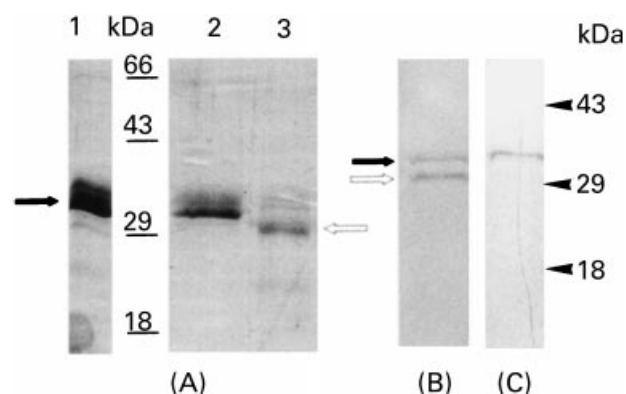


Fig. 3. Autocatalytic processing of ProDer p 1. Purified ProDer p 1 was activated for 10 min at 60 °C at different pH. Digestion products were analysed by SDS-PAGE and proteins were detected by Coomassie blue staining (panel A), Lane 1: purified ProDer p 1. Lane 2: ProDer p 1 activated at pH 7, Lane 3: ProDer p 1 activated at pH 4. ProDer p 1 activated at pH 4 was also detected by immunoblotting (panel B) with rabbit polyclonal serum raised against Der p 1 peptide 245–267 or by lectin staining (panel C) with *Galanthus nivalis* agglutinin (GNA). Black arrows and white arrows indicate the position of native and matured ProDer p 1, respectively.

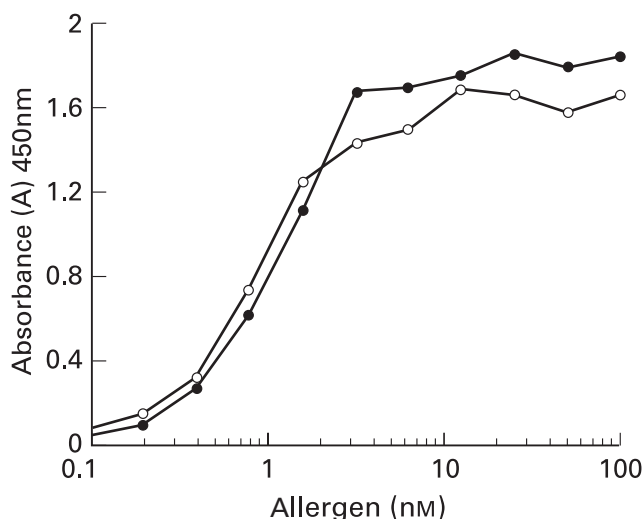


Fig. 4 Immune recognition of ProDer p 1 and Der p 1 by monoclonal antibodies directed to Der p 1. Reactivity of Der p 1 (●) and ProDer p 1 (○) towards monoclonal antibodies was assayed in a two-site ELISA. Both allergens were used at the same concentration.

fluorogenic substrates previously used for assaying proteolytic activity of Der p 1 [20,21]. The activity was compared to that of mature Der p 1. Der p 1 displayed enzymatic activity towards both substrates, the natural allergen cleaving totally fluorogenic molecules within 4 min in our

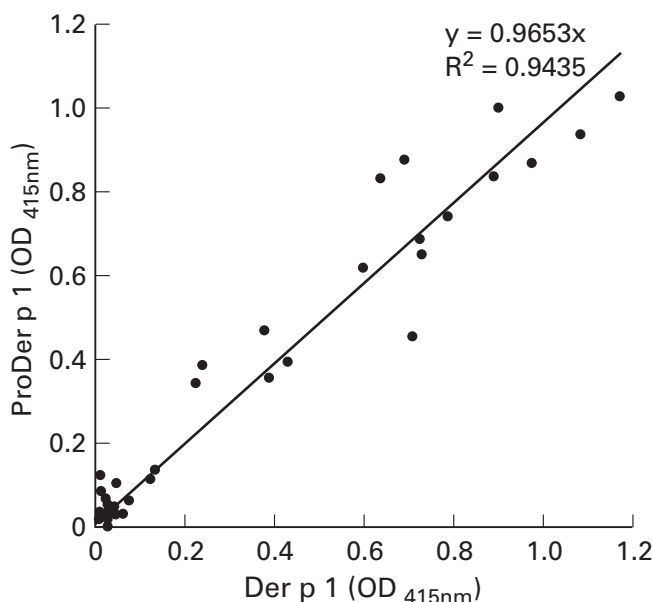


Fig. 5 IgE-binding characteristics of ProDer p 1 and Der p 1. 42 sera (diluted 1 : 8) from mite-allergic and nonallergic patients were tested for the presence of specific IgE directed to Der p 1 and ProDer p 1 in ELISA. Plates were coated with 500 ng/well of purified Der p 1 or ProDer p 1. Results are expressed as OD_{415nm} values.

experimental conditions. ProDer p 1, used at the same concentration, was totally inactive in our assays.

IgG and IgE-binding properties of ProDer p 1

The correct folding of purified ProDer p 1 was assessed in a two-site ELISA with two Der p 1 specific monoclonal antibodies. As shown in Fig. 4, ProDer p 1 and Der p 1 displayed similar reactivity suggesting that the recombinant allergen was correctly folded.

Sera of 42 acarian-allergic patients, showing reactivity with house dust mite allergens in the RAST assay, were selected to analyse the diagnostic potential of ProDer p 1. These sera were tested in a direct ELISA wherein immunoplates were directly coated with Der p 1 or ProDer p 1. IgE titre determinations clearly showed a close correlation of IgE-reactivity with both allergens, indicating that ProDer p 1 has very similar IgE-binding characteristics compared with Der p 1 ($R^2 = 0.9479$, $r < 0.0001$) (Fig. 5). IgE antibodies from four mite allergic patients with high RAST values, did not react with both allergens.

Discussion

Der p 1, as all cysteine proteinases, is most probably synthesized *in vivo* as a preproenzyme, an inactive precursor. After cleavage of the preregion, the remaining proenzyme undergoes further processing to mature forms. To date, there are few studies that described the expression of correctly folded mature Der p 1 [14–16], the expression of recombinant mature Der p 1 in yeast being more appropriate for maintaining the IgE-binding reactivity of the allergen [15]. In our hands however, and whatever the expression systems used (bacteria, yeast, insect or CHO cells), direct expression of a mature form of Der p 1 was not possible without the propeptide, suggesting that the proregion is probably essential for the allergen processing.

By replacing the Der p 1 signal sequence with the gp67 signal sequence and using the *Drosophila* inducible expression system, we succeeded in over-expressing ProDer p 1 in S2 cells. The recombinant molecule was efficiently secreted into the culture medium in high yield and its purification was achieved in three straightforward steps.

Because of the presence of two putative N-glycosylation sites in the ProDer p 1 amino acid sequence, we compared the reactivity of natural Der p 1 and recombinant ProDer p 1 to some lectins. Glycan analysis showed that the recombinant allergen was modified by high mannose-type sugar chains whereas the natural allergen did not react with any of the five different lectins used in our study, suggesting that natural Der p 1 might be a nonglycosylated allergen. This result is at variance with previously published data [22],

although similar results were obtained with matured ProDer f 1 produced in a baculovirus expression system [23,24].

The IgG- and IgE-binding activities of ProDer p 1 were compared to those of natural Der p 1 in ELISA assays. The results clearly indicate that the antigenic properties are preserved in the recombinant protein and that ProDer p 1 maturation is not necessary for immune recognition with specific IgE or monoclonal antibodies. Moreover, purified ProDer p 1 displayed a better reactivity towards IgE than a mature form of Der p 1 expressed in yeast [15]. These results were not observed for recombinant ProDer f 1, expressed in baculovirus system [23,24]. Consequently, the propeptide is likely essential for correct folding of Der p 1, in a manner similar to that seen for subtilisin E [25]. This is the first time that a recombinant form of Der p 1 is correctly folded to conserve totally its IgE epitopes.

It must be pointed out that, in our study, four out of 42 patient sera, showing strong reactivity with natural acarian allergens in RAST, did not react either with natural Der p 1 or with ProDer p 1. These results are well correlated with previous studies that mentioned that Der p 1-specific IgEs are detected in 70–80% of allergic patient sera [2,3].

Enzymatic activity assays performed with a fluorogenic substrate clearly led to the conclusion that ProDer p 1 is totally inactive in our experimental conditions. This expected observation, already observed for ProDer f 1 and other cysteine proteinases [24,26–29], could be likely explained by the pro-region insertion into the Der p 1 active site which renders the allergen inactive. In order to determine whether ProDer p 1 is able to process itself in an autocatalytic process, the pro-enzyme was activated at low pH at 60 °C, in conditions similar for the processing of some cysteine proteinase precursors (papain, caricain, cathepsin B and K) [26–29]. The stimulation of the maturation processing by cysteine demonstrates that the conversion of ProDer p 1 to Der p 1 is a cysteine proteinase-dependent event. However, no proteolytic activity was detected after ProDer p 1 maturation. There are several possible reasons for this: (i) mature Der p 1 could be unstable in our experimental maturation conditions; (ii) the propeptide cleavage could be incorrect, leading to the presence of some excedentary N-terminal amino acids of the propeptide or to the truncation of the N-terminal part of the Der p 1 mature sequence; (iii) mature Der p 1 could be autoproteolytically degraded.

It is now well established that the biological activity of allergens may promote the immunological conditions necessary for specific sensitization towards allergens [30]. In the case of Der p 1, the potent allergenicity is at least due to its proteolytic activity, which could lead to general increase in IgE synthesis (increasing Th2 immune response) [9–12,31]. Consequently, the inactive ProDer p 1 might be useful for the immunotherapeutical treatment of allergies by a 'Th2 to Th1 switch mechanism'. The use of enzymatically inactive

allergens in immunotherapy was previously suggested in two recent studies. Indeed, the data showed that mice immunization with enzymatically active papain induces an IgG1 response associated to mast cell degranulation whereas inactive form of papain blocked with the inhibitor E-64 appeared to induce a desensitization [32]. Furthermore, it was shown that inactivation of Der p 1 reduces its ability to modulate the immune response against an irrelevant antigen [33]. Attempt to revert the Th2/Th1 balance with purified ProDer p 1 are currently under study in a murine model wherein animals are sensitized after immunizations with purified natural enzymatically active Der p 1.

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