



Cloning and characterization of a major allergen of the house dust mite, *Dermatophagoides pteronyssinus*, homologous with glutathione *S*-transferase

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Abstract

A major allergen of the house dust mite, *Dermatophagoides pteronyssinus*, has been identified and characterized from a λ gt11 cDNA library of the mite. IgE antibodies from the sera of allergic patients that recognise the cloned polypeptide bind to an ~26 kDa polypeptide on a Western blot of reduced mite polypeptides. Nucleotide sequencing of the clone revealed a 219 amino acid open reading frame encoding a protein with a derived molecular mass of 25 589 Da and a *pI* of 6.3. Comparison of the deduced amino acid sequence with amino acid sequence databanks revealed a strong homology with glutathione *S*-transferases. The nucleotide sequence of the clone displayed a strong homology with the active glutathione binding site of glutathione transferases and contained all but one of the 19 positionally conserved amino acid residues found in glutathione transferases. The cloned polypeptide was expressed in *Escherichia coli* and affinity-purified on glutathione agarose.

Keywords: Glutathione *S*-transferase; IgE antibody; Allergy; (*D. pteronyssinus*)

1. Introduction

House dust mites of the genus *Dermatophagoides* are a major cause of asthma, among sensitized individuals world-wide [1–3]. The spectra of allergens recognised by IgE antibodies in the sera of many mite-allergic subjects is heterogeneous and 32 IgE-binding components have been identified in *D. pteronyssinus* extracts by immunoblotting [4]. Several of these allergens are recognised by a high percentage (40%–90%) of allergic individuals and are considered to be 'major' allergens [4]. It has been difficult to isolate individual allergens for study, both because of the difficulty of obtaining sufficient quantities of mite for extraction and the small amounts of allergen present in extracts. Isolation of cDNA clones of *D. pteronyssinus* has resulted in the characterization of some individual mite allergens: *Der p* I [5]; *Der p* II [6]; and the partial nucleotide sequence of a clone encoding a 14–15 kDa

mite allergen [7]. The clone encoding the protein corresponding to *Der p* I, however, only bound IgE antibodies weakly from allergic sera [5]. Use of IgE plaque immunoassay [8], enables the direct detection of IgE-binding clones from a cDNA library constructed in a lambda vector. In this study we report the identification of an IgE-binding ~26 kDa allergen of *D. pteronyssinus* by IgE plaque immunoassay and the determination of its nucleotide and derived amino acid sequences. The derived sequence displays strong homology with glutathione *S*-transferases from a number of species. The cloned polypeptide was expressed and affinity purified from an *Escherichia coli* lysate on glutathione agarose.

2. Materials and methods

2.1. Sera

Serum samples from mite skin prick test-positive patients were obtained from the Allergy Clinic, Royal

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North Shore Hospital (Sydney, Australia), Dr. D. Bass, Concord Hospital (Sydney, Australia) and Dr. R. Baker, Macquarie St, Sydney, Australia. Cord serum was obtained from the Department of Obstetrics, Royal North Shore Hospital.

2.2. Preparation of mite extract

0.5 g of *D. pteronyssinus* lyophilized bodies (Commonwealth Serum Laboratories (CSL), Parkville, Victoria, Australia) were extracted as previously described [9]. Extracts were pooled and concentrated using a 2000 Da cut-off filter in an Amicon ultrafiltration unit. Final protein concentrations ranged between 5 and 9 mg/ml, determined using the Pierce BCA protein determination kit (Pierce, Rockford, IL USA). Extracts were stored at -20°C .

2.3. Isolation of poly(A)⁺ mRNA and Northern hybridization

Total RNA from live mites (Commonwealth Serum Laboratories, Parkville, Victoria, Australia) snap-frozen with liquid nitrogen, was extracted with 5.5 M guanidium thiocyanate, 25 mM sodium citrate 0.5% (v/v), sodium laurylsarcosine (pH 7.0) (18 ml/g tissue)) and separated by isopycnic centrifugation in cesium trifluoroacetate gradients (Pharmacia LKB Biotechnology, Uppsala, Sweden) as described [10]. Poly(A)⁺ mRNA was prepared from total RNA, run through denaturing gels and transferred to nylon membranes as described [11]. cDNA inserts were radioactively labelled in agarose with [³²P]dCTP (Amersham, Buckinghamshire, UK; > 3000 Ci/nmol) using a random primed DNA labelling kit (Boehringer Mannheim, Germany). Labelled inserts were separated from unincorporated radioactive nucleotides by passing the solution through a Sephadex G-50 fine (Pharmacia LKB Biotechnology, Uppsala, Sweden) spun column [12] and hybridization of labelled inserts to Northern blots was as described [11].

2.4. Isolation of IgE-binding cDNA clones from a *D. pteronyssinus* cDNA library in λ gt11

A λ gt11 cDNA library prepared from *D. pteronyssinus* poly(A)⁺ mRNA [7] was immunoscreened [8] with a pool of sera from mite-allergic subjects, having the full spectrum of allergen recognition [4]. IgE-binding cDNA clones were identified using alkaline-phosphatase-labelled anti-human anti-IgE (Kallestad, Chaska, Minnesota, USA). 17 IgE-binding cDNA clones were plaque purified and high titre stocks ($> 10^{10}$ pfu/ml) prepared by plate confluent lysis. Bacteriophage stocks were prepared by CsCl block gradient

centrifugation and DNA was prepared from CsCl stock using the formamide method [13]. cDNA inserts were isolated from the λ gt11 clones by restriction digestion of 50 μg of DNA with *Eco*RI and subsequent electrophoresis in 0.7% low melting temperature agarose (Bio-Rad, Richmond, CA, USA) gels. The gels were stained with ethidium bromide solution and DNA visualized with ultraviolet exposure [12] and the cDNA inserts excised. cDNA clones were sorted by DNA dot-hybridization [14] using cDNA inserts labelled with ³²P with a random primer labelling kit (Boehringer Mannheim, Germany).

2.5. Preparation of *E. coli* lysates

Lysogens of IgE-binding λ gt11 cDNA clones were prepared grown and induced as described [13]. Cell pellets from 100 ml culture volumes were resuspended in 1.0 ml of buffer (100 mM Tris-HCl, pH 7.4, 10 mM EDTA, 1 mM PMSF) then snap-frozen with liquid nitrogen. Cells were thawed and 1.0 ml of lysis buffer (50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 100 mM NaCl) added and the preparation sonicated [15].

2.6. IgE-radioimmunoassay

Nitrocellulose (Bio-Rad laboratories, Hercules, CA, USA) discs (6 mm disc, 0.45 μm pore size) were impregnated with (i) lysates of induced lysogens of λ clones and (ii) mite extract (80 μg of total lysate protein and 20 μg of mite protein per disc, as determined by the Pierce BCA protein determination kit, Pierce, Rockford, IL USA). These discs were then tested with mite-allergic sera in a radioimmunoassay. Bound IgE antibodies were detected with ¹²⁵I-labelled anti-human anti-IgE (Biolone Sydney, Australia). Results were expressed as a % uptake by the disc of the total labelled anti-IgE added (30 000 cpm per disc). Cord serum was used as a non-IgE containing serum control in all experiments and an induced lysate of a non-recombinant λ gt11 lysogen was used to determine background levels of IgE antibodies binding to *Escherichia coli* proteins. A positive uptake was taken as a value greater than three times the cord value and/or greater than twice the uptake achieved with the control non-recombinant λ gt11 lysogen.

2.7. Cyanogen bromide (CNBr)-activation and coupling of lysates to activated paper discs

CNBr (Sigma, St. Louis, MO, USA) activation of 5 mm Whatman 540 paper discs was carried out as previously described [16] and protein coupling achieved with the following modifications: 0.2 M NaHCO₃ (pH 8.3) was mixed with an equal volume of the *E. coli*

lysates and added to CNBr-activated paper discs which were incubated on a platform rocker at room temperature overnight. Discs were then washed three times with 0.1 M NaHCO₃, blocked for 5 h with 0.1 M ethanolamine (pH 9.0) and washed (three times for 15 min each wash) with 0.018 M sodium acetate buffer (pH 4.0) followed by washing (three times for 15 min each wash) with phosphate-buffered saline (0.05 M NaH₂PO₄, pH 7.2, 0.15 M NaCl) containing 0.05% (v/v) Tween 20. The coupled discs were stored in phosphate-buffered saline with 0.1% NaN₃ (w/v) at 4°C.

2.8. Sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis and Western blotting of mite proteins

Extracts of mite for SDS polyacrylamide gel electrophoresis were prepared as follows: (1) reduced (5.0% (v/v) mercaptoethanol, 10 min/ room temperature) (2) reduced (5.0% (v/v) mercaptoethanol) and heated (100°C/10 min) and (3) heated (100°C/10 min), and all treatments were carried out in sample buffer (120 mM Tris, 8.0% SDS (w/v), 20.0% glycerol (v/v), 2.0% bromophenol blue (w/v), pH 6.8). 390 μg of total mite protein was separated on gradient (9–27%) SDS-polyacrylamide gels and electroblotted to nitrocellulose membranes (Schleicher and Schuell, Germany, 0.1 μm pore size). 4 mm blot strips were then probed with patient's sera (diluted 1/10 in phosphate-buffered saline, 0.1% Tween 20 (v/v), 0.5% bovine serum albumin (w/v) and 0.1% NaN₃ (w/v)) overnight. Strips were then washed with 0.05% Tween 20 (v/v) in phosphate-buffered saline and probed overnight with ¹²⁵I-labelled anti-human anti-IgE (Biolone), 80 000 cpm per strip, diluted as above for sera. The following day strips were again washed and exposed overnight to a phosphor storage screen (Molecular Dynamics, Sunny Vale, CA, USA). Results were analysed using a PhosphorImager, Imagequant programme (Molecular Dynamics, Sunny Vale, CA, USA).

2.9. Immunocharacterization of cDNA clones

IgE antibodies identifying recombinant proteins were prepared by incubating *E. coli* lysate-coupled 50 mm CNBr discs with sera (diluted 1:10 in phosphate-buffered saline, 0.1% (v/v) Tween 20, 0.5% (w/v) bovine serum albumin and 0.1% (w/v) NaN₃) and incubating overnight at room temperature on a platform rocker. Discs were washed 3 times for 15 min each wash with 0.05% (v/v) Tween 20 in phosphate-buffered saline and antibodies were eluted from the discs by incubating the discs for 10 min with elution buffer (0.1 M glycine-HCl, pH 2.5, 1% (w/v) BSA). The eluted antibodies were immediately neutralised with 3 M Tris (pH 8.8) and stored with 0.1% (v/v)

NaN₃. Eluted antibodies were used to directly immunoprobe strips of *D. pteronyssinus* Western blots.

2.10. Nucleotide sequencing of cDNA clone

cDNA inserts were ligated with *Eco*RI digested, phosphatased vector, pGEMEX-1 [17] using the manufacturer's protocol (Promega, Madison, USA). Recombinant plasmids were used to transform competent *E. coli* JM109 cells. Plasmid mini-preps [18] were used as template for sequencing. Sequencing was carried out using a dye-labelled primer cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were carried out in a Perkin Elmer Cetus thermal cycler using the T3 and SP6 dye-labelled primers and reaction products were analysed using an Applied Biosystems Model 373A DNA Sequencer (Applied Biosystems, Foster City, CA, USA). Subsequent sub-cloning of cDNA restriction fragments for the generation of a full overlapping sequence in both directions was carried out in the plasmid vector pGEM-7zf (+) (Promega) as described above for pGEMEX-1. Sequencing in this vector was carried out using the T7 and SP6 dye-labelled primers. Sequence data was prepared using DNASISTM (Hitachi Software Engineering, Yokohama, Japan) and sequence analyses performed using Geneworks (Intelligenetics, Mountain View, CA, USA). Nucleotide and amino acid sequence homology searches were carried out at the NCBI using the BLAST network service, accessed via the Australian National Genome Information Service (ANGIS).

2.11. Affinity-purification of cloned polypeptides using glutathione agarose

The pGEMEX-1 plasmid construct was transformed into *E. coli* JM109 (DE3) competent cells and the cloned polypeptide expressed as described by the manufacturer (Promega). Cell lysates were prepared as described earlier, with the exception that lysates were thawed and resuspended in an equal volume of 0.1 M NaH₂PO₄ (pH 7.2), 0.3 M NaCl. Recombinant polypeptides were affinity-purified from *E. coli* lysates with 10 ml of S-linked glutathione agarose (Sigma, St. Louis, MO) and the bound polypeptides eluted with 50 mM Tris (pH 9.6), 5 mM reduced glutathione as previously described [19,20]. Eluted recombinant polypeptides were concentrated in an Amicon ultrafiltration unit with 2000 Da cut-off filters and the resulting solution was further concentrated in micro10 concentrators (Amicon). The concentrated polypeptide solution was electrophoresed on gradient (8–27%) Phastgel (Pharmacia) and stained using the rapid Coomassie stain method.

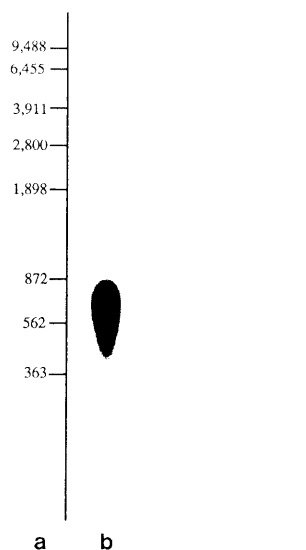


Fig. 1. Northern analysis of the cDNA insert of mite sub-clone p Dp 15. The cDNA insert of p Dp 15 was radioactively labelled with ^{32}P and used to probe a Northern blot of mite poly(A) $^{+}$ mRNA. (a) RNA size markers (bases) and (b) hybridization profile for p Dp 15 cDNA insert.

3. Results

3.1. Characterization of IgE-binding clones from a mite λ gt11 cDNA library

17 IgE-binding cDNA clones from the mite λ gt11 cDNA library were identified and plaque purified by IgE plaque immunoassay using a pool of mite-allergic sera. The 17 IgE-binding clones were screened in a dot hybridization assay using a cDNA insert from a mite cDNA clone encoding an IgE binding 14–15 kDa protein previously identified in our laboratory [7]. Non-hybridizing clones were selected for further study. The radioactively labelled cDNA insert of one of the non-hybridising clones, designated λ Dp 15, gave a diffuse band centred at 800 bases on hybridisation to a Northern blot of mite poly(A) $^{+}$ mRNA (Fig. 1). The cDNA insert of λ Dp 15 was excised with the restriction endonuclease *Eco*RI and was determined to be approximately 800 base pairs.

3.2. IgE radioimmunoassays

IgE radioimmunoassays were carried out to determine the frequency of recognition of the protein encoded by λ Dp 15 among mite-allergic subjects whose

sera gave a positive result in radioimmunoassays against mite extract. 77 of the 193 sera tested (40%) gave a positive result with the *E. coli* lysate of λ Dp 15.

3.3. Immunological characterization

To locate a common polypeptide recognised by all positive sera, strips of Western blots of unreduced mite extract were probed with the positive sera. However, no binding of IgE antibodies to any common blotted proteins was observed. When strips of Western blots of reduced mite extracts were probed with the positive sera, IgE antibodies were found to react with an ~26 kDa polypeptide band.

A serum highly reactive against the *E. coli* lysate of λ Dp 15 was reacted with lysates coupled to CNBr-activated paper discs (see experimental procedures). Antibodies that bound to the λ Dp 15 recombinant protein were eluted and used to probe strips cut from a Western blot of reduced total mite extract and bound antibodies were detected with ^{125}I -labelled anti-human anti-IgE. Eluted IgE antibodies were found to bind to a mite polypeptide of molecular mass ~26 kDa (Fig. 2). Antibodies eluted from an induced non-recombinant λ lysogen coupled to a CNBr-activated paper disc, displayed no binding to any of the mite proteins on the Western blot (Fig. 2). To differentiate between the ~26 kDa protein being studied and *Der p* I, a protein of molecular mass 25 371 Da, Western blots of (1) non-heated, reduced and (2) heated, reduced extracts were probed with antibodies eluted from the λ Dp 15 induced lysogen coupled to the CNBr activated paper disc. Western blots of reduced (5.0% mercapto-ethanol (v/v)), unheated extracts of mite suggest *Der p* I has a molecular mass of ~25 kDa, however, under reducing and heating (100°C/10 min) conditions *Der p* I appears to have a molecular mass of ~30 kDa [21]. Under both conditions the antibodies bound to a band of ~26 kDa, suggesting that this protein was different to *Der p* I.

3.4. Sequence analysis of p Dp 15

Sequencing reactions of p Dp 15 using the SP6 and T3 dye-labelled primers revealed the location of a *Cl*aI restriction site at nucleotide number 244 (Fig. 3). The *Cl*aI site was used to create two sub-clones in the vector pGEM-7ZF (+), for further sequencing. The second round of sequencing revealed an *N*siI restriction site at nucleotide number 488 (Fig. 3). The *N*siI site was used to create further sub-clones, for sequencing in pGEM-7ZF (+). The construction of these sub-clones enabled the determination of the complete overlapping sequence in both the 5' to 3' and 3' to 5' directions (Fig. 4). The complete nucleotide sequence is 869 nucleotides long with a methionine residue at

beginning at nucleotide 52. Three bases upstream from the start of the methionine codon is an adenosine and in 80% of cases studied the nucleotide 3 bases upstream from the start codon is adenosine [22] suggesting that this methionine residue is the start codon. The predicted open reading frame begins at nucleotide number 52 and ends at nucleotide number 709 and encodes a protein of 219 amino acids with a derived molecular weight of 25 589 Da and a pI value of 6.3.

3.5. Prediction of antigenic determinants

Hydropathicity profiles for the derived amino acid sequence of p Dp 15 were determined using Genewords (Intelligenetics). Both the Hopp and Woods

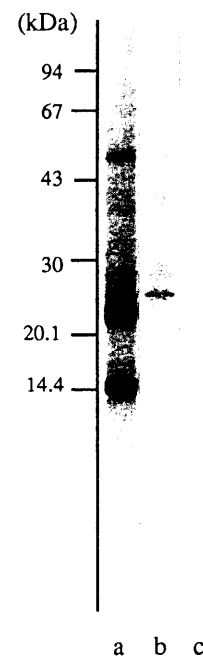


Fig. 2. Localization of the native mite protein corresponding to the protein encoded by p Dp 15. Numbers on the left represent the positions of the protein molecular weight markers run on the 9–27% gradient SDS-polyacrylamide gel. Reduced extracts of mite (treatment 1, Materials and Methods) were run on a gel and transferred to nitrocellulose as described in Experimental procedures. Strips of nitrocellulose were probed with (a) serum f428 from a mite-allergic subject, (b) serum f428 antibodies eluted from an *E. coli* lysate of λ Dp 15 coupled to a CNBr-activated paper disc and (c) serum f428 antibodies eluted from an *E. coli* lysate of a non-recombinant λ lysogen control.

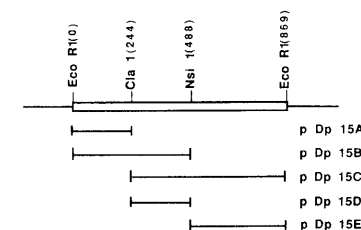


Fig. 3. Restriction map of p Dp 15 cDNA insert. The cDNA insert from mite clone λ Dp 15 was sub-cloned into the plasmid vector p GEMEX-1 and cycle sequenced. Nucleotide sequence data of the entire insert was obtained by constructing the sub-clones indicated; p Dp 15A, p Dp 15B, p Dp 15C, p Dp 15D and p Dp 15E in the plasmid vector pGEM-7ZF (+). All sub-clones were sequenced at least twice in both directions.

[23] and Kyte and Doolittle [24] algorithms were applied (Fig. 5). The following regions of hydrophilicity, and hence potential antigenicity, were identified; residues 10–15, 26–55, 61–71, 80–107, 117–132 and 176–215 (Kyte and Doolittle) and residues 27–34, 39–52, 66–70, 80–108, 118–132 and 180–203 (Hopp and Woods).

3.6. Homology with other protein sequences

Comparison of the sequence of p Dp 15 with sequences of other sequenced mite allergens; *Der p* I [5], *Der p* II [6] and a 15 kDa protein [7], revealed that it was a different protein. A homology search of the nucleotide and deduced amino acid sequence of p Dp 15 against current databases revealed a 50% homology with the Yb subunits of rat and mouse glutathione S-transferases, class Mu. 19 positionally conserved amino acid residues have been previously identified throughout the sequences of glutathione transferases [20]. The sequence of p Dp 15 contains 18 of the 19 conserved amino acids. The first 80 residues of glutathione transferases, containing the highly conserved exon 4 region, have been identified as containing the binding region for glutathione [20, 25]. When compared with the highly conserved region encoded by exon 4 of glutathione S-transferases [20], 24 out of 32 residues (75%) of p Dp 15 were identical to the rat glutathione transferase exon 4 (Fig. 6). Two of the un-matched residues, number 52 (asparagine) and number 70 (methionine), represented a single nucleotide change in the codons encoding the amino acids.

3.7. Affinity purification of the recombinant polypeptide

The *E. coli* lysate of p Dp 15 was affinity purified with glutathione agarose. The eluted material was elec-

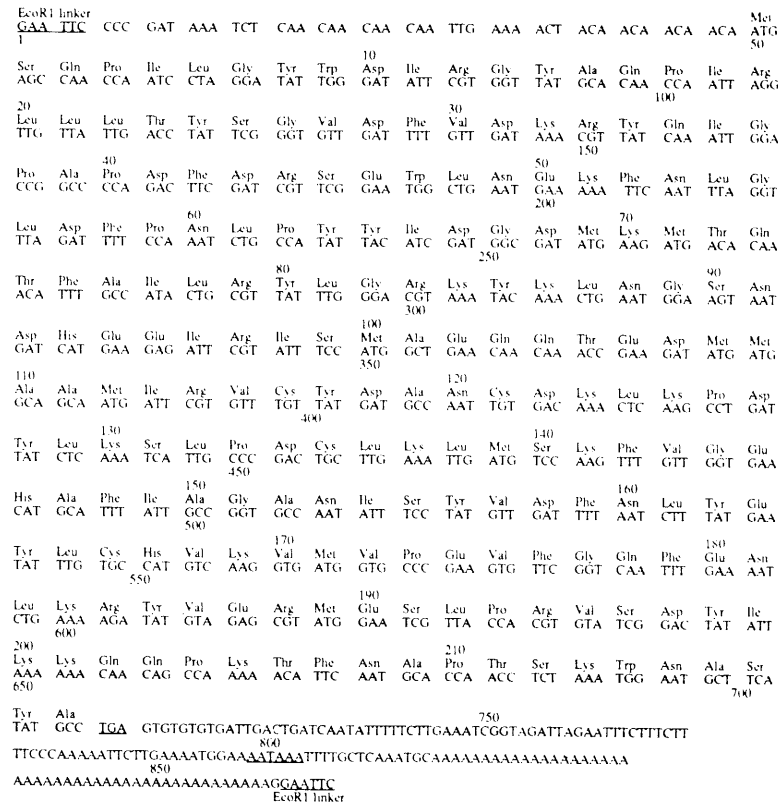


Fig. 4. Nucleotide and deduced amino acid sequence of the cDNA insert of p Dp 15. The open reading frame of the amino acid sequence encoded by p Dp 15 begins at nucleotide 52 and ends at nucleotide 709. The putative polyadenylation signal (AATAAA), the stop signal (TGA) and the EcoRI linkers (GAATTC) are underlined. The insert has an (A)₄₆ poly(A) tail.

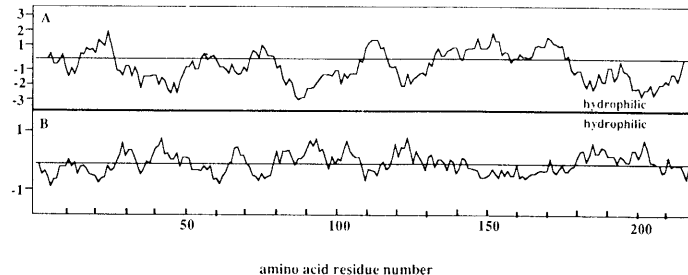


Fig. 5. Hydropathicity plots of the deduced amino acid sequence of the insert of p Dp 15. Hydropathicity was calculated by the Geneworks programme using two hydropathicity algorithms: Kyte and Doolittle (A) and Hopp and Woods (B). Calculated regions of hydrophobicity are negative (below the line) in the Kyte and Doolittle representation and positive (above the line) in the Hopp and Woods representation.

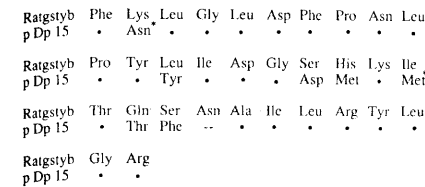


Fig. 6. Comparison of the amino acid sequence of rat glutathione-S-transferase Yb (Ratgstyb) subunit exon 4 with the deduced amino acid sequence of p Dp 15. The sequence of Ratgstyb exon 4 reported in the Genbank database is shown aligned with a homologous region of the amino acid sequence of p Dp 15. Dots (·) indicate identical residues and the two asterisked (*) residues represent a single nucleotide substitution resulting in a different amino acid. The dashed line indicates where the sequence was stretched to obtain the best fit.

trophoresed on gradient Phast gels and stained with Coomassie blue. A polypeptide band of 55 kDa was visualised, corresponding in size to the 29 kDa gene 10 product of pGEMEX-1 [26] fused to the 26 kDa cloned polypeptide. The position of the affinity-purified polypeptide corresponded in size to the expressed fusion polypeptide in the p Dp 15, induced total cell lysate, not observed in the negative control non-recombinant, induced, pGEMEX-1 cell lysate. Western blots of affinity-purified fusion polypeptides were probed with sera from patients with IgE antibodies to λ Dp 15. The serum IgE antibodies bound to the 55 kDa fusion polypeptide.

4. Discussion

In this paper we have described the identification and characterisation of a cDNA clone that encodes an ~26 kDa allergen of the house dust mite, *Der-matophagoides pteronyssinus*, recognised by 40% of sera from mite-allergic patients. IgE antibodies eluted from an *E. coli* lysate of λ Dp 15 bound to an ~26 kDa protein on a Western blot of reduced mite proteins. The ~26 kDa polypeptide was distinguished from *Der p* 1, another important mite allergen with a molecular mass of ~25 kDa, by the fact that its mobility was unchanged following reduction and heating, unlike *Der p* 1 whose apparent molecular mass changes from 25 kDa to 30 kDa on heating of a reduced sample.

Nucleotide sequencing of the cDNA insert of p Dp 15 revealed an open reading frame encoding a protein with a derived molecular mass of 25 589 kDa. This agreed with the earlier molecular mass of ~26 kDa, determined by immunological analysis after SDS-polyacrylamide gel electrophoresis and blotting. A search of current nucleotide and protein databanks revealed a 50% homology between the sequence of p Dp 15 and

the Yb subunits of rat and mouse glutathione S-transferase. Closer inspection revealed that the sequence of p Dp 15 contained 18 of the 19 positionally conserved glutathione transferase amino acid residues previously identified [20]. The sequence of p Dp 15 is 75% homologous with the highly conserved exon 4 region of rat glutathione transferase Yb subunit. The exon 4 region of glutathione transferases has been previously shown to be involved in the binding of glutathione [20,25]. The presence of the positionally conserved glutathione transferase residues in the sequence of p Dp 15 and the strong homology between the purported glutathione binding site and the sequence of p Dp 15 strongly indicates that p Dp 15 encodes a mite glutathione transferase.

Glutathione S-transferases are cytosolic proteins composed of two subunits about 24–28 kDa each [20]. The most extensively studied glutathione S-transferases, those from the rat, are encoded by a multigene family and different combinations of subunits form different classes of transferase with a number of different species within each class. It is possible that the same complexity may be observed with glutathione S-transferases from different species [27]. The diffuse band observed when Northern blots of mite poly(A)⁺ mRNA were probed with the cDNA insert from p Dp 15, supports the notion that the cloned polypeptide is from a family of related proteins, as observed for other glutathione S-transferases. Examination of the hydropathicity plots of the protein encoded by p Dp 15 revealed no evidence of a leader sequence in the form of an N-terminal sequence of hydrophobic amino acid residues. This would seem to correspond to the lack of leader sequences observed on cytosolic proteins. Further evidence to suggest that the protein encoded by p Dp 15 is a glutathione S-transferase and, most likely, a Yb subunit, is provided by its pI value. The pI of the protein encoded by p Dp 15 is 6.3 which is similar to the pI of 6.8 for rat liver glutathione S-transferase Yb subunit [28].

There are three common hydrophilic regions predicted by the two hydropathicity algorithms we employed; residues 80–107, 117–132 and 176–215 (Kyte and Doolittle) and residues 80–108, 118–132 and 180–203 (Hopp and Woods). Experiments are currently being undertaken to locate the antigenic determinants of the 26 kDa mite glutathione S-transferase.

The homology observed between the sequence of p Dp 15 and the glutathione transferases, in particular the 75% homology with exon 4, suggested that the expressed polypeptide would bind glutathione. It was possible to purify the expressed p Dp 15 polypeptide on glutathione agarose and the affinity-purified polypeptide had a molecular mass of 55 kDa, consistent with the expected size of a fusion protein.

It has previously been reported that glutathione

S-transferase is one of the major proteins that stimulates an IgE response during infections with *Schistosoma mansoni*. Capron et al. [29] have suggested that allergic responses to environmental allergens may reflect immune activation ordinarily initiated by parasites. Although there is, as yet, little evidence to support this speculation, the implication of glutathione S-transferases in IgE-mediated reactions to both *Shistosoma* species and *D. pteronyssinus* may be an example of a connection between IgE-mediated responses to helminths on the one hand and allergens on the other.

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