

RAPID PUBLICATION

Sequence analysis of cDNA coding for a major house dust mite allergen, *Der f I*

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Summary

A cDNA clone coding for *Der f I*, a major allergen from the house dust mite *Dermatophagoides farinae* has been isolated and sequenced. It codes for a putative 18-residue signal peptide, an 80-residue proenzyme region, and a 223-residue mature protein with a derived molecular weight of 25 191. The deduced amino-acid sequence shows significant homology to other cysteine proteases in the proregion as well as in the mature protein. Sequence alignment of the mature *Der f I* protein with the homologous allergen *Der p I* from the related mite *D. pteronyssinus* revealed a high degree of homology (81%) between the two proteins, as predicted by previous sequencing at the protein level. In particular, the residues comprising the active site of these enzymes and the cysteine residues were conserved. A potential *N*-glycosylation site was present at an equivalent position in both mite allergens. It is anticipated that the availability of recombinant *Der f I* will facilitate epitope mapping studies and studies of T-cell function in mite allergy by providing high levels of pure allergen.

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Introduction

The association between allergy to mites of the genus *Dermatophagoides* and allergic diseases such as asthma, rhinitis and atopic dermatitis has been recognized for over two decades [1,2]. Two allergens from the predominant mite species, *D. pteronyssinus* and *D. farinae*, have been found to be particularly important [3–6]. One of these major mite allergens, designated *Der p I*, a 25-kD protein excreted in mite faeces [7] has over 50% of the IgE response directed against it in up to 60% of allergic sera [8–10]. Equivalent responses occur for the homologous allergen, *Der f I* from *D. farinae*, which shows extensive crossreactivity in binding IgE and IgG. Studies on purified *Der p I* and *Der f I* have demonstrated that these allergens possess both crossreacting and species-specific epitopes [11].

Recently, the molecular cloning of mite allergens was initiated to aid in their definition and characterization and provide reagents for clinical use and epitope analysis. This approach resulted in the isolation of a cDNA clone

coding for *Der p I* which contained a 0.8-kb cDNA insert [12]. Sequence analysis revealed that the 222 amino-acid residue mature recombinant *Der p I* protein showed significant homology with a group of cysteine proteases including actinidin, papain, cathepsin H and cathepsin B. Based on comparison with these other cysteine proteases, it appears that *Der p I* has a preproenzyme sequence which includes and extends beyond the 23 amino-acid residues of the truncated proenzyme sequence of the current cDNA clone [13,14].

When expressed in *E. coli* as a recombinant protein, *Der p I* has been found to have lower reactivity to IgE compared with that of the native protein [15]. This is probably an indication that the protein is not folding into its correct conformation. It is known that proenzyme regions are involved in protein folding, as demonstrated in a recent study which showed that the prosequence of the enzyme subtilisin can guide the folding of subtilisin to an active conformation when added to inactive mature subtilisin expressed in *E. coli* [16]. It is probable that the synthesis of active *Der p I* also requires the proregion for correct folding of the mature protein.

We now report the isolation and characterization of a cDNA clone coding for the allergen *Der f I*. This *Der f I*

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cDNA clone contains a 1.1-kb cDNA insert encoding a typical signal peptide, a proenzyme region and the mature *Der f I* protein. It is anticipated that the expression of the prepro-*Der f I* protein in *E. coli* will produce a recombinant protein of greater solubility, stability and antigenicity than recombinant *Der p I*. If successful, the availability of high yields of soluble *Der f I* allergen and antigenic derivatives will facilitate the development of reagents for mapping B- and T-cell epitopes and performing other serological and cellular studies.

Materials and methods

Dermatophagoides farinae culture

Mites were purchased from Commonwealth Serum Laboratories, Parkville, Australia.

Construction of the *D. farinae* cDNA lambda gtlI library

Polyadenylated mRNA (10 µg) was isolated from 5 g live *D. farinae* as described for *D. pteronyssinus* [17] and 2 µg used to synthesize cDNA by the RNase H method [18] using a kit (Amersham International, Aylesbury, U.K.). After the addition of EcoRI linkers (New England Biolabs, Beverly, U.S.A.) the cDNA was ligated to alkaline-phosphatase treated lambda gtlI arms (Promega Biotec, Madison, U.S.A.). The ligated DNA was packaged and plated in *E. coli* Y1090 (r⁻) to produce a library of 2×10^4 recombinants.

Isolation of *Der f I* cDNA clones from the *D. farinae* cDNA lambda gtlI library

Screening of the library was performed by hybridization with two probes comprising the two *Der p I* cDNA BamHI fragments (1–348 and 349–857 generated by BamHI digestion of a derivative of the *Der p I* cDNA which has had two BamHI restriction sites inserted between amino acid residues –1 and 1 and between residues 116 and 117 by site-directed mutagenesis [12, Trudinger *et al.*, unpublished]. The probes were radiolabelled with ³²P by nick translation. Phages were plated at 20 000 p.f.u. per 150 mm Petri dish and plaques were lifted onto nitrocellulose (Schleicher and Schull, Dassel, Germany) and denatured and baked [18]. Prehybridizations were performed for 2 hr at 42°C in 50% formamide/5 × SSCE (with 0.01 M EDTA)/1 × Denhardt's/poly C (0.1 mg/ml)/poly U (0.1 mg/ml) with hybridization overnight at 42°C at 10⁶ c.p.m./ml and washed at high stringency.

Isolation of DNA from gtlI f I cDNA clones

Phage DNA from gtlI f I clones was prepared by a rapid isolation procedure [12] and subcloned [19].

DNA sequencing and analysis

To obtain clones for DNA sequence analysis, the gtlI f I phage DNA was digested with EcoRI restriction enzyme (Pharmacia, Uppsala, Sweden) and ligated to EcoRI-digested M13-derived sequencing vectors mp18 and mp19 [20]. Transformation was carried out using *E. coli* TG-1 and sequencing was performed by the dideoxynucleotide chain termination method [21] using the sequenase version 2.0 DNA sequencing kit (U.S.B., Cleveland, U.S.A.).

Sequence analysis was performed using the Mac VECTOR Software (IBI, New Haven, U.S.A.). Hydrophobicity plots were computed with the software according to the Hopp Woods [22] algorithm using a six-residue window.

Polymerase chain reaction (PCR)

PCR was performed by the Taq DNA polymerase method [23] using the TaqPak kit (Biotech International, Bentley, U.S.A.) and the conditions recommended by the supplier, with 10 ng of target DNA and 10 pmol of lambda gtlI primers (New England BioLabs, Beverly, U.S.A.).

RESULTS

Isolation of *Der f I* cDNA clones

Two clones containing the major mite allergen *Der f I* were isolated from the *D. farinae* cDNA lambda gtlI library by their ability to hybridize with both of the *Der p I* cDNA probes (nucleotides 1–348 and 349–857). This approach was adopted because amino-acid sequencing had shown high homology (80%) between these two allergens [15]. Digestion of the lambda gtlI f I clone DNA with EcoRI restriction enzyme to release the cDNA insert, produced three *Der f I* cDNA EcoRI fragments; one approximately 800 bases long and a doublet approximately 150 bases long. The *Der f I* cDNA insert was also amplified from the phage DNA by the PCR resulting in a PCR product of approximately 1.1-kb. Each *Der f I* cDNA fragment was cloned separately into the M13-

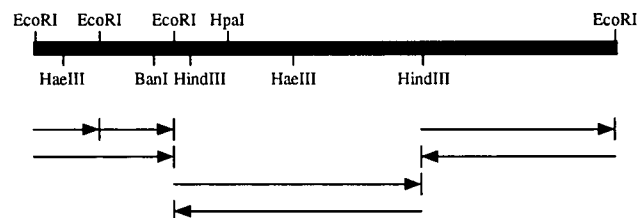


Fig. 1. Restriction map of the cDNA insert of clone gtlI f I and the strategy of DNA sequencing. Arrows indicate directions in which sequences were read.

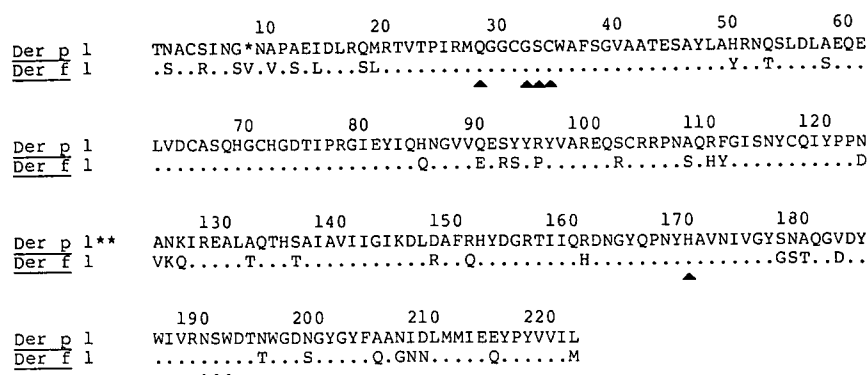


Fig. 3. Composite alignment of the amino-acid sequences of the mature *Der p* I [12] and *Der f* I proteins. The numbering above the sequence refers to *Der p* I. * Denotes the gap that was introduced for maximal alignment. The symbol • is used to indicate that the amino-acid residue of *Der f* I at that position is identical to the corresponding amino acid residue of *Der p* I. The arrows indicate those residues making up the active site of *Der p* I. ** *Der p* I amino-acid sequence from residues 134–143 as predicted by nucleotide sequence, revised June 1990 [unpublished data].



Fig. 4. Comparison of amino-acid sequence in the pre- and propeptide regions of *Der f* I with those of rat cathepsin H, rat cathepsin L, papain, aleurain, CP1, CP2, rat cathepsin B [13], CTLA-2 α , CTLA-2 β , MCP [33], *Der p* I [12] and actinidin [34]. Gaps, denoted by dashes, were added for maximal alignment. ** Denotes conserved amino-acid residues which are shared by greater than 80% of the proenzymes. * Residues which are conserved in greater than 55% of the sequences. The symbol • is used to denote semiconserved equivalent amino acids which are shared by greater than 90% of the proenzyme regions (see text).

addition, the immediate flanking sequence of this ATG codon (CAAAAATG) conforms to the Kozak consensus sequence (CCACCATG) for the eukaryotic translation initiation sites [24]. *Der f I* has the crucial purine (most often A) at position -3 (i.e. three nucleotides upstream from the initiator codon) and has a cytosine in position -5, a position of C predominance.

Amino-acid sequence analysis

The amino-acid sequence predicted by nucleotide analysis is shown in Fig. 2. As shown in the composite alignment of the amino-acid sequence of mature *Der p I* and *Der f I* (Fig. 3), high homology (81%) was observed between the two proteins. In particular, the residues making up the active site of *Der p I*, based on those determined for papain, actinidin, cathepsin H, and cathepsin B [12], are also conserved in the *Der f I* protein. The residues are glutamine (residue 29), glycine, serine and cysteine (residues 33–35), histidine (residue 171) and asparagine, serine and tryptophan (residues 191–193), where the numbering refers to *Der f I*. All of the seven cysteine residues present in *Der p I* are conserved in *Der f I* (Fig. 3). The predicted mature *Der f I* amino-acid sequence contains a potential N-glycosylation site (Asn-Thr-Ser) at position 53–55 which is also present as Asn-Gln-Ser at the equivalent position in *Der p I* [12].

Analysis of the predicted amino-acid sequence of the entire *Der f I* cDNA insert has shown that, as for other cysteine proteases (Fig. 4), the *Der f I* protein has pre- and proform intermediates. As previously mentioned, the methionine residue at position -98 is presumed to be the initiation methionine. This assumption is based on the fact that firstly, the 5' proximal end sequence from residues -98 to -81 is composed predominantly of hydrophobic amino-acid residues (72%), which is the characteristic feature of signal peptides [25]. Secondly, the lengths of the presumptive pre- (18 amino-acid residues) and propeptides (80 residues) are similar to those for other cysteine proteases (Fig. 4). Most cysteine proteases examined have about 120 preproenzyme residues (of which an average of 19 residues form the signal peptide) with cathepsin B the smallest with 80 [13]. *Der f I* falls within this range with a total of 98 preproenzyme residues.

By following the method for predicting signal-sequence cleavage sites outlined previously [25], it is proposed that cleavage from the pre-*Der f I* sequence for proenzyme formation occurs at the signal peptidase cleavage site lying between Ala (-81) and Arg (-80). Thus, the sequence from residues -98 to -81 codes for the putative leader peptide while the proenzyme moiety of *Der f I* begins at residue Arg (-80) and ends at residue Glu (-1).

DISCUSSION

The isolation and sequencing of a cDNA clone coding for *Der f I* reported here is the first sequence determination of the complete primary translation product of a Group I house dust mite allergen (the 25 kD allergens *Der p I*, *Der f I* and *Der m I*, from the mite species *D. pteronyssinus*, *D. farinae* and *D. microceras*, respectively). The complete sequence of the *Der f I* primary translation product has been determined and appears to code for a 321 amino-acid residue protein containing a putative signal peptide (18 residues), pro-peptide region (80 residues) and mature enzyme region (223 residues). This agrees with similar studies involving the cloning of the cDNA for other cysteine proteases which have established the sequence of the N-terminal extension peptides of the mature enzyme, i.e. a signal peptide and propeptide [13].

A comparison of mature *Der f I* sequence with that of mature *Der p I* by sequence alignment (Fig. 3) showed that the degree of homology between these two mite allergens was very high. In particular, the crucial active site residues were completely conserved and all of the cysteine residues were homologous in *Der f I*. Conserved cysteine residue pairs (31, 71) and (65, 103), where the numbering refers to *Der p I*, are apparently involved in disulphide bond formation on the basis of the assumed similarity of the three-dimensional structure of *Der p I* and *Der f I* to that of papain and actinidin which also have an additional disulphide bridge. The fifth and final cysteine residue for which there is a homologous cysteine residue in papain and actinidin is the active site cysteine (residue 35 in *Der f I*). It is likely that the two extra cysteine residues present in *Der p I* and *Der f I* (residues 4 and 117; *Der p I* numbering) may be involved in forming a third disulphide bridge.

The potential N-glycosylation site in *Der p I* is also present at the equivalent position in *Der f I* with conservation of the crucial first and last residues of the tripeptide site (Asn-x-Ser/Thr) [12]. However, the degree of glycosylation of *Der f I* and *Der p I* has yet to be determined, although carbohydrates including mannose, galactose, N-acetylglucosamine and N-acetylgalactosamine have been reported in purified preparations of these mite allergens [9,26].

Given the degree of homology over the first 30 N-terminal amino-acid residues between mature *Der p I* and *Der m I* (70%) and mature *Der f I* and *Der m I* (97%), with the *Der m I* residues determined by conventional amino-acid sequencing [27,28], it is probable that the full mature *Der m I* sequence will confirm an overall 70–80% homology between the Group I mite allergens. High homology between the proenzyme moieties of *Der p I* and *Der f I* (91%) over the residues -23 to -1 and the

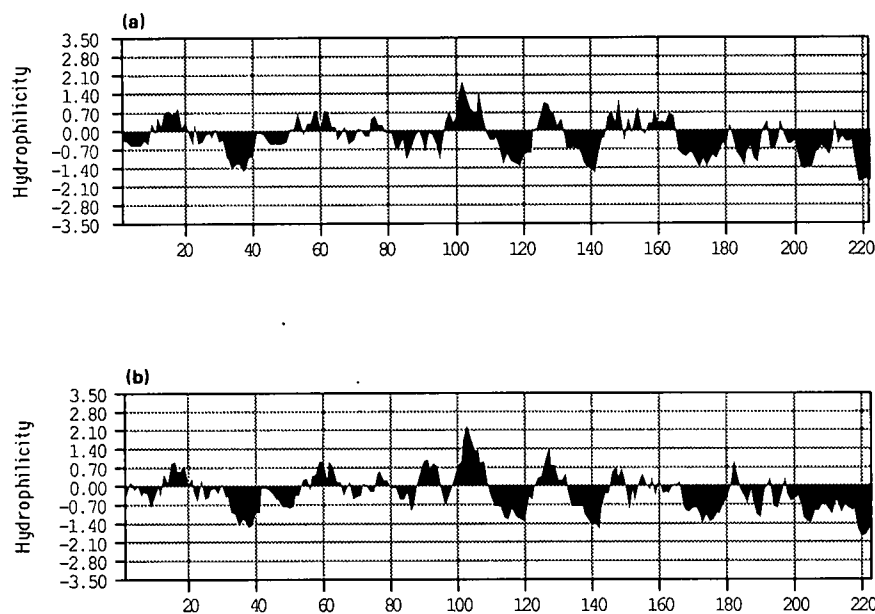


Fig. 5. Hydrophilicity plots of the *Der p I* (a) and the *Der f I* (b) mature proteins using the Hopp-Woods algorithm [22] computed with the Mac VECTOR sequence analysis software (IBI, New Haven, U.S.A.) using a six-residue window. Positive values indicate relative hydrophilicity and negative values indicate relative hydrophobicity.

structural analysis of *Der f I* presented here suggests that the Group I allergens are likely to have N-terminal extension peptides of the mature protein of homologous structure and, at least for the propeptide, composition.

The open reading frame for *Der f I* consists of a typical leader sequence [25] probably encompassing the first 18 residues. However, the following sequence encodes a proenzyme region with significant sequence homology to the pro-regions of other cysteine protease [13]. Alignment of the proenzyme regions of *Der f I* and a number of other cysteine proteases (Fig. 4) indicated that these proregions share a number of very conserved residues as well as some residues which were present in over half of the sequences. This homology was increased if conservative amino acids such as Val, Ile and Leu (small hydrophobic residues) or Arg and Lys (positively charged residues) were regarded as identical. The *Der f I* proregion possessed six out of the seven highly conserved amino acids and all the residues at sites of conservative changes. The homology at less conserved sites was lower. Homology found in the propeptide, in particular the highly conserved residues, may be important when considering the function of the propeptide in the processing of these enzymes because it indicates that these sequences probably have structural and functional similarities.

With the availability of the complete amino-acid sequence of recombinant *Der f I* and *Der p I* it will now be feasible to attempt to map the epitopes recognized by both the B- and T-cell compartments of the immune system.

Highly crossreactive B-cell epitopes on *Der f I* and *Der p I* have been demonstrated using antibodies present in

mouse, rabbit and human sera [11,29]. However, species-specific epitopes have also been defined in these systems, with murine monoclonal antibodies binding predominantly to species-specific determinants [30], some 40% of rabbit anti-*Der p I* reactivity were accounted for by epitopes unique to *Der p I* [29], and some species-specific binding of antibodies from allergic humans, although the majority bind to crossreactive epitopes [30]. B-cell (and T-cell) epitopes specific for either species may provide useful diagnostic reagents for determining reactivity to the different mite species while crossreacting epitopes would be candidates for a common immunotherapeutic agent.

The sequence differences shown here between the *Der p I* and the *Der f I* proteins are primarily located in the N- and C-terminal regions as well as in an extended surface loop (residues 85–136) linking the two domains of the enzyme that includes helix D (residues 127–136), as predicted from the secondary and tertiary structures of papain and actinidin [31]. The surface location of these residues is supported by the hydrophilicity plots of *Der p I* and *Der f I* (Fig. 5) which illustrate the predominantly hydrophilic nature of this region that predicts surface exposure. This loop region also contains two species-specific B-cell epitopes recognized by a rabbit anti-*Der p I* serum in a recent study by Greene *et al.* [32]. Two of the crossreactive epitopes defined in these experiments (located in regions comprising residues 34–47 and 60–71) are completely conserved between *Der p I* and *Der f I*, while the majority of residues in a third crossreactive epitope-containing region (residues 166–194) were conserved. The structural basis for the species-specific reactivity of the two *Der p I*-specific antigenic regions may be

that they appear to be located on a surface loop and are thus perhaps less highly conserved than those regions associated with defined secondary structures or the active site cleft which characterize the regions containing the crossreactive epitopes.

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