

SEQUENCE ANALYSIS OF cDNA CODING FOR
A MAJOR HOUSE DUST MITE ALLERGEN, *Der p 1*
Homology with Cysteine Proteases

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It has long been recognized that the allergy to mites of the genus *Dermatophagoides* is associated with diseases such as asthma, rhinitis, and atopic dermatitis (1, 2). In this regard, the species *D. pteronyssinus* and *D. farinae* predominate and many studies have been performed to identify the allergens they produce. Studies on *D. pteronyssinus* have, for example, demonstrated that at least six major allergens are of clinical importance (3, 4). One of the major mite allergens, designated *Der p 1*, has been purified and shown to react with anti-mite IgE antibodies in up to 80% of allergic sera (3-5). This allergen, reportedly a glycoprotein of mol wt 27×10^3 , appears to be an excretory product associated with fecal particles (6).

Currently, the diagnosis and immunotherapy of the house dust mite allergy is based on the use of crude mite preparations (7). Developments in this field have been hampered by the small or variable quantities of mite allergens present in the extracts. To help overcome these limitations, and to facilitate further studies, the molecular cloning of mite allergens is being investigated in our laboratory. To date, a cDNA clone coding for the allergen *Der p 1* has been isolated and shown to contain a 0.8-kb cDNA insert (8). We now report the sequence analyses of the *Der p 1* cDNA clone and the similarity between its inferred amino acid sequence and the group of cysteine proteases.

Materials and Methods

Dermatophagoides pteronyssinus Culture. Mites were purchased from Commonwealth Serum Laboratories, Parkville, Australia.

Isolation of Genomic DNA. Extraction of mite genomic DNA was carried out by a modified guanidium-HCl/cesium chloride (CsCl) method (9). 10 g of live mites were

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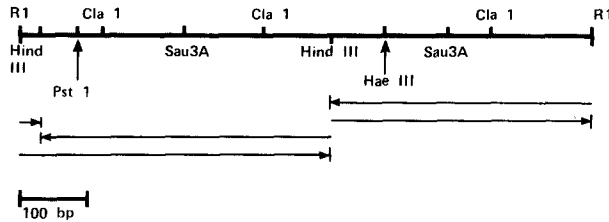


FIGURE 1. Restriction map of the cDNA insert of clone λ gt 11 *p 1* (13T) and the strategy of DNA sequencing. Arrows indicate directions in which sequences were read.

ground in the presence of liquid nitrogen and sand to form a paste. 8 ml of 6 M guanidine hydrochloride in 0.1 M sodium acetate buffer pH 5.2 were then added and the mixture was homogenized and spun at 10,000 rpm for 30 min in a Sorvall SS34 rotor. The supernatant was collected and layered onto a CsCl pad (5 ml of 4.8 M CsCl in 10 mM EDTA) and centrifuged at 37,000 rpm for 16 h at 15°C in a SW41 T1 rotor (Beckman Instruments, Inc., Fullerton, CA). The DNA band at the interphase was collected and diluted 1:15 in 10 mM Tris HCl/1 mM EDTA buffer, pH 8.0. Banding of genomic DNA in CsCl was carried out by the standard method.

Isolation of DNA from λ gt 11 *p 1* cDNA Clone. Phage DNA from λ gt 11 *p 1* clone was prepared by a rapid isolation procedure. Clarified phage plate lysate (1 ml) was mixed with 270 μ l of 25% wt/vol polyethylene glycol (PEG 6000) in 2.5 M NaCl and incubated at room temperature for 15 min. The mixture was then spun for 5 min in a microfuge (Eppendorf, Federal Republic of Germany), and the supernatant was removed. The pellet was dissolved in 100 μ l of 10 mM Tris/HCl pH 8.0 containing 1 mM EDTA and 100 mM NaCl. This DNA preparation was extracted 3 times with phenol/chloroform (1:1) and the DNA was precipitated by ethanol.

DNA Hybridization. Nucleic acid was radiolabeled with 32 P by nick translation. DNA samples were digested with appropriate restriction enzymes using conditions recommended by the supplier. Southern blots were prepared using Zeta-Probe membranes (Bio-Rad Laboratories, Richmond, CA). Prehybridization, hybridization, and posthybridization washes were carried out according to the manufacturers recommendations (bulletin 1234, Bio-Rad Laboratories).

Cloning and DNA Sequencing. To clone the 0.8-kb cDNA insert from clone λ gt 11 *p 1* into plasmid pUC8, phage DNA was digested with Eco RI restriction enzyme and then ligated to Eco RI-digested pUC8 DNA and used to transform *Escherichia coli* JM83. The resulting recombinant plasmid was designated as pHDM 1.

To obtain clones for DNA sequence analysis, the cDNA insert was isolated from pHDM 1 and ligated to M13-derived sequencing vectors mp18 and mp19 (10). Transformation was carried out using *E. coli* JM107 and sequencing was performed by the dideoxynucleotide chain termination method (11).

Amino Acid Sequence Homology Determination. Homology between the derived amino acid sequence of *Der p 1* and other proteins was determined by comparing the sequence with those present in the National Biomedical Research Foundation protein library using the MATCH algorithm (12) of the Protein Identification Resource suite of programs.

Results

DNA Sequence Analysis. A clone expressing the major mite allergen *Der p 1* was isolated from a mite cDNA library by its ability to react with an anti-*Der p 1* antiserum and to hybridize with oligonucleotides constructed on the basis of known amino acid sequence data (8, 13). The nucleotide sequence of the cDNA insert from this clone, λ gt 11 *p 1*, was determined using the sequencing strategy shown in Fig. 1. The complete sequence was shown to be 857 bases long and included a 69-base-long 5' proximal end sequence, a coding region for the entire native *Der p 1* protein of 222 amino acids with a derived molecular weight of

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-23                                     -10
AAA AAC CGA TTT TTG ATG AGT GCA GAA GCT TTT GAA CAC CTC AAA ACT 48
Lys Asn Arg Phe Leu Met Ser Ala Glu Ala Phe Glu His Leu Lys Thr
                                     -1 1
CAA TTC GAT TTG AAT GCT GAA ACT AAC GCC TGC AGT ATC AAT GGA AAT 96
Gln Phe Asp Leu Asn Ala Glu Thr Asn Ala Cys Ser Ile Asn Gly Asn
10                                     20
GCT CCA GCT GAA ATC GAT TTG CGA CAA ATG CGA ACT GTC ACT CCC ATT 144
Ala Pro Ala Glu Ile Asp Leu Arg Gln Met Arg Thr Val Thr Pro Ile
30                                     40
CGT ATG CAA GGA GGC TGT GGT TCA TGT TGG GCT TTC TCT GGT GTT GCC 192
Arg Met Gln Gly Gly Cys Gly Ser Cys Trp Ala Phe Ser Gly Val Ala
50
GCA ACT GAA TCA GCT TAT TTG GCT CAC CGT AAT CAA TCA TTG GAT CTT 240
Ala Thr Glu Ser Ala Tyr Leu Ala His Arg Asn Gln Ser Leu Asp Leu
60                                     70
GCT GAA CAA GAA TTA GTC GAT TGT GCT TCC CAA CAC GGT TGT CAT GGT 288
Ala Glu Gln Glu Leu Val Asp Cys Ala Ser Gln His Gly Cys His Gly
80
GAT ACC ATT CCA CGT GGT ATT GAA TAC ATC CAA CAT AAT GGT GTC GTC 336
Asp Thr Ile Pro Arg Gly Ile Glu Tyr Ile Gln His Asn Gly Val Val
90                                     100
CAA GAA AGC TAC TAT CGA TAC GTT GCA CGA GAA CAA TCA TGC CGA CGA 384
Gln Glu Ser Tyr Tyr Arg Tyr Val Ala Arg Glu Gln Ser Cys Arg Arg
110                                     120
CCA AAT GCA CAA CGT TTC GGT ATC TCA AAC TAT TGC CAA ATT TAC CCA 432
Pro Asn Ala Gln Arg Phe Gly Ile Ser Asn Tyr Cys Gln Ile Tyr Pro
130
CCA AAT GCA AAC AAA ATT CGT GAA GCT TTG GCT CAA CCA CAG CGC TAT 480
Pro Asn Ala Asn Lys Ile Arg Glu Ala Leu Ala Gln Pro Gln Arg Tyr
140                                     150
TGC CGT CAT TAT TGG ACC ATC AAA GAT TTA GAC GCA TTC CGT CAT TAT 528
Cys Arg His Tyr Trp Thr Ile Lys Asp Leu Asp Ala Phe Arg His Tyr
160
GAT GGC CGA ACA ATC ATT CAA CGC GAT AAT GGT TAC CAA CCA AAC TAT 576
Asp Gly Arg Thr Ile Ile Gln Arg Asp Asn Gly Tyr Gln Pro Asn Tyr
170                                     180
CAC GCT GTC AAC ATT GTT GGT TAC AGT AAC GCA CAA GGT GTC GAT TAT 624
His Ala Val Asn Ile Val Gly Tyr Ser Asn Ala Gln Gly Val Asp Tyr
190                                     200
TGG ATC GTA CGA AAC AGT TGG GAT ACC AAT TGG GGT GAT AAT GGT TAC 672
Trp Ile Val Arg Asn Ser Trp Asp Thr Asn Trp Gly Asp Asn Gly Tyr
210
GGT TAT TTT GCT GCC AAC ATC GAT TTG ATG ATG ATT GAA GAA TAT CCA 720
Gly Tyr Phe Ala Ala Asn Ile Asp Leu Met Met Ile Glu Glu Tyr Pro
220                                     222
TAT GTT GTC ATT CTC TAAACAAAAAGACAATTTCTTATATGATTGTCACCTAATTTATT 778
Tyr Val Val Ile Leu
TAAATCAAAATTTTAGAAAATGAATAAATTCATTCACAAAAATTAATAAAAAAAAAAAAAAAAA 841
AAAAAAAAAAAAAAAAAAAA 857

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FIGURE 2. Nucleotide and predicted amino acid sequence of cDNA λ gt 11 p1. Numbers to the right are nucleotide positions whereas numbers above the sequence are amino acid positions. Positive amino acid residue numbers correspond to the sequence of the mature excreted *Der p* 1 beginning with threonine. Negative sequence numbers refer to the proposed transient pre- and proenzyme forms of *Der p* 1. The arrows indicate the beginning of the proposed proenzyme sequence and the mature *Der p* 1, respectively. Residues -15 to -13 enclosed by an open box make up the proposed cleavage for the proenzyme formation (see text), and the dashed residues 52-54 represent a potential *N*-glycosylation site. The termination TAA codon and the adjacent polyadenylation signal are underlined. Amino acid residues 1-41, 79-95, 111-142, and 162-179 correspond to known tryptic peptide sequences determined by conventional amino acid sequencing analysis. These sequence data have been submitted to the EMBL/GenBank Data Libraries under accession number Y00641.

25,371, an 89-base long 3' noncoding region and a poly(A) tail of 33 residues (Fig. 2).

The assignment of threonine residue at position 1 as the NH₂-terminal amino acid of *Der p* 1 was based on data obtained by NH₂-terminal amino acid sequencing of the pure protein isolated from mite excretions (13). The predicted amino acid sequence matched with data obtained by amino acid sequence analysis of



FIGURE 3. Composite alignment of the amino acid sequences of *Der p 1*, papain (15), actinidin (16), cathepsin H (17), and cathepsin B (18) (pre- and proregions omitted). Gaps were added to maximize similarity and the arrows indicate those residues making up the active site of papain as deduced by x-ray crystallograph (15).

the NH₂-terminal region as well as with internal sequences derived from analyses of tryptic peptides (Fig. 2). The complete mature protein is coded by a single open reading frame terminating at the TAA stop codon at nucleotide position 736–738. At present, it is not certain whether the first ATG codon at nucleotide position 16–18 is the translation initiation site, since the immediate flanking sequence of this ATG codon (TTGATGA) showed no homology with the Kozak consensus sequence (ACCATGG) for the eukaryotic translation initiation sites (14). In addition, the 5' proximal end sequence does not code for a typical signal peptide sequence (see below).

Amino Acid Sequence Analysis. The amino acid sequence predicted by nucleotide analysis is shown in Fig. 2. A protein data-base search revealed that the *Der p 1* amino acid sequence showed homology with a group of cysteine proteases. As shown in the composite alignment of the amino acid sequence *Der p 1* and four cysteine proteases (Fig. 3), significant homology was observed in the amino and COOH-terminal regions of the proteins. Sequence homology analysis revealed that *Der p 1* protein showed 29% homology with actinidin, 24% homology with papain, 28% homology with cathepsin H, and 21% homology with cathepsin B. The two crucial amino acid residues (cysteine and histidine) known to be in the active sites of papain, actinidin, cathepsin H, and cathepsin B (15–17) are also conserved in the *Der p 1* protein (as cysteine residue 34 and histidine residue 170) in addition to other residues that make up the active site (Fig. 2).

Previous cDNA studies have shown that lysosomal cathepsins B, a mouse

macrophage protease and a cysteine protease from an amoeba have transient pre- and proform intermediates (18–20), and inspection of the amino acid sequence at the 5'proximal end of the λ gt11 *p* 1 cDNA clone suggests that *Der p* 1 may be similar. First, the hydrophilicity plot (21) of the sequence preceding the mature protein sequence lacks the characteristic hydrophobic region of a signal peptide (22) and second, an Ala-X-Ala sequence, the most frequent sequence preceding the signal peptidase cleavage site (23, 24), is present at positions –13, –14, –15 (Fig. 2). Therefore, we propose that cleavage between pro-*Der p* 1 sequence and the pre-*Der p* 1 sequence occurs between Ala (–13) and Phe (–12). Thus, pro-*Der p* 1 sequence begins at residues Phe (–12) and ends at residues Glu (–1). The amino acids residues numbered –13 to –23 would then correspond to a partial signal peptide sequence.

Southern Blot Analysis of Genomic DNA. When the 857-bp cDNA insert was radiolabeled and hybridized against a Southern blot of Eco RI-digested genomic DNA from house dust mite, hybridization to bands of 1.5, 0.5, and 0.35 kb was observed (data not shown). As shown in the restriction enzyme map of the cDNA insert (Fig. 1), there was no internal Eco RI site and the multiple hybridization bands observed suggest that *Der p* 1 is coded by a noncontiguous gene. The results also showed little evidence of gene duplication since hybridization was restricted to fragments with a total length of 2.4 kb.

Discussion

By using molecular and immunological techniques, we have isolated a cDNA clone coding for the complete sequence of mature protein of a major mite allergen, *Der p* 1. ~40% of the mature *Der p* 1 protein sequence had been determined by the conventional amino acid sequencing of NH₂-terminal and tryptic peptides and showed no discrepancy with those predicted from the DNA sequence.

A comparison of mature *Der p* 1 sequence with sequences of the cysteine proteases, papain, actinidin, cathepsin B, and cathepsin H by sequence alignment revealed that the degree of homology between the four sequences was very high in both the NH₂-terminal and the COOH-terminal regions. In particular, the residues making up the active site of *Der p* 1, based on those determined for papain, are highly conserved. The residues are glutamine (residue 28), glycine serine and cysteine (residues 32–34), histidine (residue 170) and asparagine, serine, and tryptophane (residues 190–192). The central areas of all these proteins, however, show little homology with *Der p* 1 or themselves, which makes the functional significance of this region unclear. This homology shared between *Der p* 1 and cysteine proteases of both plant and animal origins is a novel observation and in this regard we have recently demonstrated cysteine protease activity in both the growth medium of mites as well as in purified preparations of *Der p* 1 (our unpublished observations).

Comparative studies of the structures of homologous proteins have shown that three-dimensional structures are evolutionary more conserved than primary amino acid sequences. Hence, by adopting a knowledge-based approach (25), it will be possible to construct a three-dimensional model of the *Der p* 1 using the known three-dimensional structures of papain and actinidin (15, 16). These data,

together with epitope mapping studies, will now be used to design novel molecules for immunotherapy and or immunodiagnosis.

By analogy with the known sequences of other cysteine proteases such as cathepsins B and H (18–20), it appears that *Der p 1* is an enzyme that has transient pre-/proenzyme and proenzyme forms. The sequence upstream of that coding for the known mature protein (NH₂-terminal, threonine) does not resemble a typical leader peptide and at positions (–13 to –15) there is a signal peptidase cleavage site Ala-X-Ala typical of these proteins (23, 24). In addition, the Ala-Phe at positions –13 and –12 are common junctional residues for preenzymes and similar proteases (23). Thus, the sequence from residues –1 to –12 code for the proenzyme moiety of *Der p 1*, and the sequences preceding these, which are incomplete in our clone, code for a partial leader peptide. Isolation and characterization of genomic DNA clones coding for *Der p 1* is being pursued and should resolve this issue. The same structural considerations should apply to the related *Der f 1* protein, which shows 50% homology with *Der p 1* in its 20 NH₂-terminal amino acid residues (8).

The predicted *Der p 1* amino acid sequence contains a potential *N*-glycosylation site (Asn-Gln-Ser) at position 52–54. Some uncertainty is apparent, however, about the degree of glycosylation of *Der p 1* since data from our laboratory (not shown) indicate no binding of radiolabeled *Der p 1* to Con A, implying little or no mannose content. This contrasts with an earlier report (26) of *Der p 1* containing mannose together with other carbohydrates.

It is likely that *Der p 1*, in contrast to the cathepsin B and H, is a secreted protein since it is found in mite excreta at a concentration of 10 mg/ml. Hence, it may have some digestive function. The possibility that its proposed proteolytic activity has some function in the induction or elicitation of allergic reactions is also intriguing. In this respect, it has been reported that clinical administration of chymopapain, or industrial and domestic exposure to papain, induces a high incidence of hypersensitivity (27).

Summary

A cDNA clone coding for *Der p 1*, a major allergen from the house dust mite *Dermatophagoides pteronyssinus*, has been sequenced. It codes for a 222 residue mature protein with a derived molecular weight of 25,371 and contains 1 potential *N*-glycosylation site. In addition, the cDNA appears to code for a 13 residue proregion, and an incomplete signal peptide. The deduced sequence shows a high degree of homology with animal and plant cysteine proteases, particularly in the region of the contact residues making up the active site. Southern analysis of genomic DNA indicates that the allergen is coded by a noncontiguous gene. These data will now facilitate epitope mapping studies.

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