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Molecular Characterisation of Group I Allergen *Eur m I* from House Dust Mite *Euroglyphus maynei*

Key Words

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Abstract

Using the polymerase chain reaction (PCR) we have amplified and cloned genomic DNA encoding the secreted group I allergen proteins from the house dust mite species *Euroglyphus maynei*, *Dermatophagoides pteronyssinus* and *D. farinae*. Affinity chromatography using a monoclonal antibody to the allergen *Der p I* was used to purify the group I protein from *E. maynei*. We present the deduced amino acid sequence of a new member of the group I house dust mite allergen family *Eur m I*. The three proteins show a high level of primary structure similarity: *Eur m I* and *Der p I* show 85% amino acid identity, and the three allergen amino acid sequences taken together show 78% identity. A potential N-glycosylation site and residues of the cysteine protease active site are also conserved between the three proteins.

A current focus in allergy research [1, 2] concerns several classes of protein from house dust mite species, implicated as elicitors of the allergic response in asthma. Using molecular biology techniques, allergen coding sequences have been cloned from *Dermatophagoides pteronyssinus* and *D. farinae* [3–6] allowing the sequence analysis and potential manipulation of these proteins. The mite species *Euroglyphus maynei* may be an important allergen source in some environments [2, 7] but has received little experimental attention because of culturing difficulties. To overcome this limitation, we have applied polymerase chain reaction (PCR) technology to amplify sequences from genomic DNA extracted from small numbers of mites, prior to cloning and sequencing.

Using the cDNA sequence obtained for *Der p I* [3] we designed two primers: one corresponding to amino acids –11 to –4 of the proenzyme form, and the other corre-

sponding to the C-terminus of the protein (fig. 1). The degeneracy of the primers and their placement within the coding region were intended to give the optimal chance of amplification from *E. maynei*, *D. pteronyssinus* and *D. farinae* genomic DNA whilst yielding maximum sequence data for the secreted group I protein (losing only 12 amino acids of the C-terminus). Genomic DNA was extracted from frozen, ground, mite bodies (with a typical yield of 30 µg DNA/100 mg mites) by proteinase K digestion in 0.5% SDS followed by phenol/chloroform extraction and ethanol precipitation. 35 amplification cycles of 95 °C for 45 s, 52 °C for 2 min and 72 °C for 2 min gave a single product which was further amplified and cloned using pUC plasmid vectors. To detect possible PCR-induced mutations, at least two recombinant clones of each construct were sequenced in both directions using Sequenase 2.0 (USB) double-stranded-dideoxy-chain-termination protocols.

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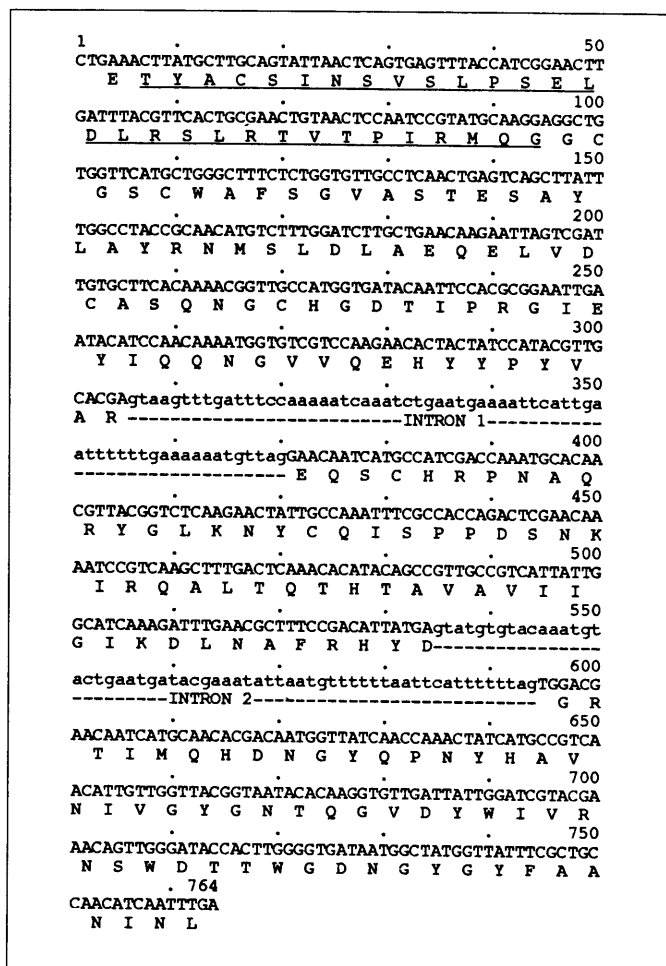


Fig. 1. Nucleotide and deduced amino acid sequence of *Eur m I* from PCR-amplified *E. maynei* DNA using primers 5'-GAAGAATT-CAAACTCARTTYGAYY 3' and 5'-ACNGAATTCGRTAYT-CYTCKATCATCA 3'. Introns are indicated in lower case. Amino acid sequence confirmed by N-terminal sequencing is underlined. These sequence data are available from EMBL under accession number X60073.

Figure 1 shows the nucleotide and deduced amino acid sequence we determined for the *E. maynei* gene. The nucleotide sequences we derived for the *Der p I* and *Der f I* genes are available on the EMBL sequence library under accession numbers X65197 and X65196, respectively. The amplified bands from all three mite species (*D. pteronyssinus*: 821 base pairs, *D. farinae*: 876 base pairs and *E. maynei*: 823 base pairs) were larger than would be predicted from the *Der p I* cDNA sequence. Our sequence data indicate that this size difference is due to the interruption of the coding regions by introns. All three genes have an intron between codons 101 and 102, and within codon 156 (fig. 1). The *Der f I* gene has an additional intron interrupt-

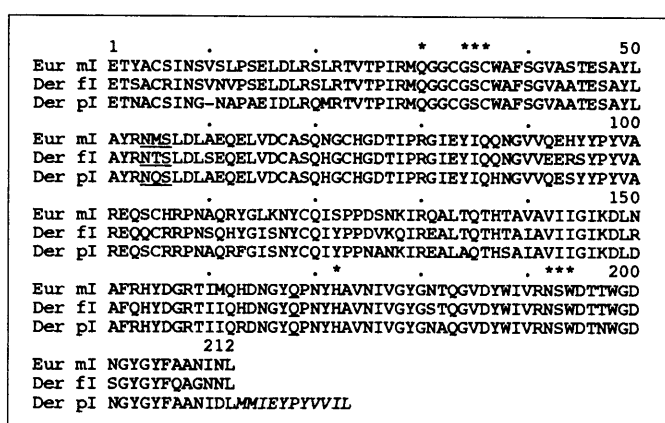


Fig. 2. Comparison of the derived amino acid sequences for *Eur m I*, *Der f I* and *Der p I*. Amino acid 2 corresponds to the N-terminus of the secreted proteins. The sequence in italics denotes the C-terminal amino acids of *Der p I* as determined by Chua et al. [3]. Amino acids marked (*) are conserved cysteine protease active site residues and the underlined region is a potential N-glycosylation site.

ing codon 80. These introns are unusually small (between 58 and 65 base pairs), but conform to the 'GT-intron-AG' splice-site consensus.

Group I protein was purified from an *E. maynei* extract using the group I cross-reactive monoclonal antibody 4CL.B8 (kindly provided by M.D. Chapman) coupled to a Sepharose 4B (Pharmacia LKB) column. Protein eluted from the column with 50% ethylene glycol, 50 mM glycine (pH 10.0) was observed to run as a single 25-kD band by SDS-PAGE, and exhibited multiple isoelectric points under isoelectric focusing between 4.7 and 5.4; similar to *Der p I* [8]. This affinity purified protein was coupled to a di-isothiocyanate membrane and subjected to solid-phase Edman degradation on a MilliGen/Biosearch 6600 ProSequencer. The N-terminal amino acid sequence of the protein was in agreement with the nucleotide sequence data (fig. 1), confirming that the PCR-derived genomic sequence from *E. maynei* does indeed encode a group I allergen. We therefore term this new member of the group I house dust mite allergens *Eur m I*.

The amino acid sequence data suggest that the N-terminal residue of the secreted protein is a threonine, as for *Der p I* and *Der f I* [3, 9].

Our derived *Der p I* and *Der f I* amino acid sequences are identical to those published or present in the EMBL 30.0 database with (using the numbering from fig. 2) the following exceptions: our *Der f I* lacks an additional methionine residue at position 22 which was assigned to the protein using N-terminal sequencing [9]; and when compared with the sequence from reference 3, our *Der p I*

shows three potential polymorphisms at amino acids 52, 97 and 180 and a DNA sequence frameshift changing residues 136–145. The reading frame we report is the same for each of the three group I allergen sequences we have obtained and is therefore probably correct.

A comparison of the deduced amino acid sequences (fig. 2) reveals 78% identity between the three proteins. Both *Eur m* I and *Der p* I, and *Eur m* I and *Der f* I show approximately 85% sequence identity with each other, and *Der p* I and *Der f* I approximately 82%. Alignment of the *Der p* I sequence with the other two also shows the presence of an additional valine residue in *Der f* I and *Eur m* I at amino acid 10 (fig. 2). The protease active-site residues inferred for *Der p* I [3], amino acids 30, 34–36, 172 and 192–194, are conserved in all three proteins. All seven cysteine residues present in *Der p* I are also conserved. Chua et al. [3] identified a possible N-glycosylation site in the *Der p* I

protein. This site (N-X-S or N-X-T, where X is any amino acid except proline) is also conserved in all three proteins at residues 54–56. This level of primary sequence similarity suggests that the three proteins will have the same gross secondary structure and enzymatic properties [10].

In conclusion, the three group I allergen sequences determined to date show a very high level of primary sequence similarity. Work can now be directed to establish the significance of this similarity in terms of epitope recognition.

Acknowledgments

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