

Cloning and Molecular Characterization of a cDNA from *Blomia tropicalis* Homologous to Dust Mite Group 3 Allergens (Trypsin-Like Proteases)

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Key Words

House dust mite · *Blomia tropicalis* · cDNA · Trypsin · Allergen

Abstract

Background: Exposure to the house dust mite *Blomia tropicalis* is increasingly being implicated as a major risk factor for asthma exacerbations in sensitized individuals. The objective of this study is to clone and characterize *B. tropicalis* allergens in order to better define their role in allergic asthma. **Methods:** A λ gt22A cDNA library was constructed from *B. tropicalis* mRNA and screened using specific DNA probes. A full-length cDNA (Bt2-3) was isolated, subcloned, and sequenced. **Results:** Sequence analysis showed that clone Bt2-3 encodes the full-length serine protease preproenzyme from *B. tropicalis*. The predicted Bt2-3 protein consists of a 15-amino-acid signal peptide, a 20-amino-acid propeptide and a mature protein of 231 amino residues. BLAST analysis of the deduced amino acid sequence showed high homology (48–54%) between clone Bt2-3 and serine proteases (group 3 allergens) from domestic dust mites. Both the serine active site (DACQGDSGGPVA; amino acids 214–225) and the histidine active site (LTAAHC; amino acids

71–76) of serine proteases were highly conserved. The estimated molecular weight of the preproenzyme is 27.5 kD and its theoretical pI is 8.7. The mature protein contains a putative tyrosine kinase phosphorylation site (amino acids 24–31) and several N-myristoylation sites. Sequence alignment shows that the serine protease active sites are highly conserved among the clinically important mite species, including *B. tropicalis*. **Conclusion:** We report the cloning and molecular characterization of a *B. tropicalis* cDNA clone encoding a trypsin-like protease, with a possible major role as an allergen. Expression and further characterization of the recombinant product will help determine the role of proteolytic enzymes in the pathophysiology of allergic asthma.

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Introduction

In Puerto Rico, *Blomia tropicalis* is the second most common mite species thriving in household dust, after *Dermatophagoides pteronyssinus* [1]. *B. tropicalis* allergens have been implicated as causes of exacerbation in sensitized patients and have been shown to induce specific IgE responses [2]. The relative abundance of this spe-

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cies in household dust and the significant percentage of asthmatic patients who are positive to *B. tropicalis* as shown by skin testing support its clinical relevance in Puerto Rico and other tropical regions of the world.

Molecular and biochemical analyses have shown that several domestic dust mite allergens have significant sequence and functional homology to proteases. Group 1 dust mite allergens (e.g., Der f 1, Der p 1) are homologous to cysteine proteases, while allergens in group 3 (e.g., Der f 3, Der p 3) are homologous to trypsin [3–5]. Der p 6 (group 6) shares high sequence homology to chymotrypsin, and Der p 9 (group 9) is homologous to collagenolytic serine proteases [6, 7]. Proteolytic allergens have also been described in cockroaches (Bla g 2) and fungi (Pen ch 13) [8, 9]. Several lines of evidence suggest that the proteolytic activity of these allergens is the major contributor to their allergenic potential [10, 11]. Proteolytic allergens have been shown to activate complement, to trigger secretion of proinflammatory cytokines by bronchial epithelial cells and eosinophils, and to disrupt the epithelial cell permeability barrier of the lung [12, 13].

To date, only 5 *B. tropicalis* allergens have been cloned and characterized at the molecular level, including Blo t 1 (homologous to cysteine proteases) [14], Blo t 5 [2, 15] (unknown function), Blo t 10 (a tropomyosin) [16], Blo t 11 (homologous to paramyosin) [17], and Blo t 13 (a fatty acid binding protein) [18, 19]. However, immunoblotting studies have shown that this mite species produces at least 18 immunodominant proteins [Montealegre F., unpubl. data]. In this report, we describe the cloning and sequence characterization of a novel *B. tropicalis* cDNA homologous to trypsin-like proteases, which comprise the group 3 dust mite allergens. These data make a significant contribution to the field of allergy and asthma as they demonstrate that *B. tropicalis* produces a trypsin-like protease likely to play a major role as an asthma-inducing allergen.

Materials and Methods

Construction of the B. tropicalis λ gt22A cDNA Library

A *NotI/EcoRI* unidirectional cDNA library was constructed using the BRL cDNA synthesis and λ -cloning/packaging systems (Gibco Brl). First and second cDNA strand synthesis was performed from poly(A)⁺ RNA using Superscript reverse transcriptase and DNA polymerase following the manufacturer's protocols. The λ gt22A vector has an IPTG-inducible promoter allowing blue/white screening of plaques. The resulting packaged library was titered by infection of Y1090 *Escherichia coli* cells. The library was amplified, retitered and screened as described below.

Screening of the Library

Using N-terminal sequence information from an immunogenic native *B. tropicalis* protein isolated in the laboratory, we designed a degenerate probe to screen a *B. tropicalis* cDNA library. Library screening was performed using standard methods [20]. In brief, Y1090 cells were infected with approximately 2×10^4 PFU per 100-mm² plate. After blotting, denaturation and cross-linking of phage DNA onto Nylon filters, positive clones were detected by incubation with biotin-labeled specific probes. Plaques corresponding to positive signals in the autoradiogram were selected and amplified by infection of *E. coli* Y1090 cells. Rescreening of the phages was performed as described until pure clones were obtained.

Subcloning and DNA Sequencing

Recombinant phage DNA was isolated from the positive clones using the Wizard Lambda Preps DNA Purification System (Promega, Madison, Wisc., USA). Complementary DNA inserts were PCR amplified using λ gt11-specific primers and subcloned into the pCR-Topo-TA vector (Invitrogen, Carlsbad, Calif., USA) following the manufacturer's protocol. Recombinant plasmids were identified by restriction analysis and sequenced in both directions using fluorescent-labeled universal primers (Cy5 AutoCycle DNA sequencing kit) in an ALFExpress automated DNA sequencing system (Pharmacia Biotech Inc., Piscataway, N.J., USA). Sequence comparisons and homology searches to GenBank protein sequences were performed using the BLAST 2.1 algorithm (www.ncbi.nlm.nih.gov/blast.cgi) [21]. DNA sequence analysis, protein translation and ClustalW alignments were performed using the MacVector version 5.0 software (IBI, New Haven, Conn., USA) and with tools available at the ExPASy molecular internet server [22]. Protein alignments were edited using GeneDoc [23].

Results

Screening of the B. tropicalis λ gt22A cDNA Library

Screening of approximately 4×10^5 recombinants from a *B. tropicalis* cDNA library identified several clones, of which one (Bt2-3) was purified to homogeneity. Insert cDNA was amplified by PCR, subcloned into pCR-TopoTA (Invitrogen) and the DNA sequence of several subclones was determined in both directions as described above.

Molecular Characterization of Positive Clones

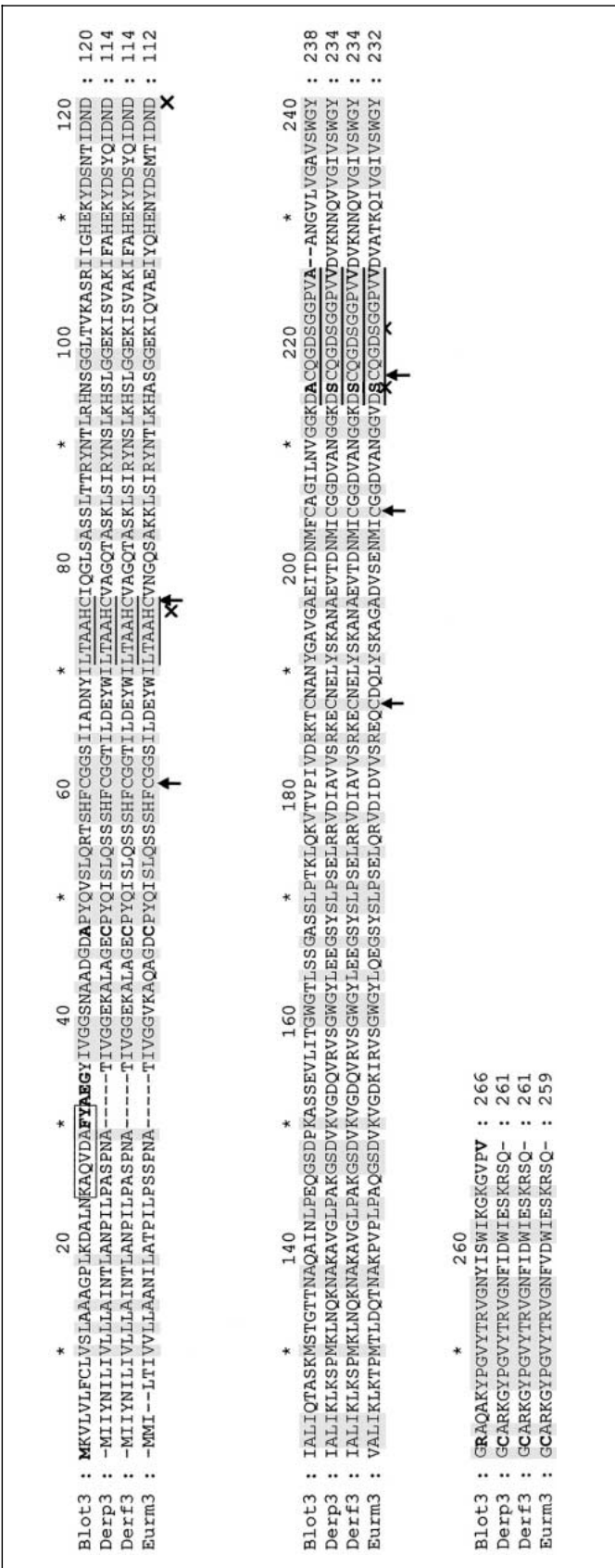
Clone Bt2-3 contained a 798-bp open reading frame, initiated by a methionine residue and terminating at the TAA stop codon. This clone included a 5' untranslated region coding for the amino acids NHLTK following two sequential stop codons. BLAST search of the NCBI protein database showed high homology to trypsin-like serine proteases at the amino acid level, including the group 3 dust mite allergens Der p 3 (49% identity, 64% positives) (accession No. P39675) [24], Der f 3 (48% identity, 66% positives) (accession No. P49275) [25], and Eur m 3 (54%

identity, 69% positives) (accession No. O97370). Also, the deduced protein sequence was homologous to a collagenolytic serine protease from *D. pteronyssinus* (49% identity, 62% positives) (accession No. AAN02510). Analysis of the amino acid sequence using the ExPASy proteomics and sequence analysis tools showed that the *B. tropicalis* trypsin consists of a 15-amino-acid signal peptide (MKVLVLFCLVSLAAA), a 20-amino-acid propeptide (GPLKDALNKAQVDAFYAEGY), and a mature 231-amino-acid polypeptide. The initiation codon of the preproenzyme is methionine, while the mature protein is initiated with the amino acid isoleucine. The theoretical pI of the *B. tropicalis* trypsin is 8.7, and the predicted molecular weight of the preproenzyme is 27.5 kD, which is similar to those of previously cloned trypsin-like dust mite allergens. The preproenzyme contains a tyrosine kinase phosphorylation site at positions 24–31 (amino acids KAQVDAFY), and twelve putative N-myristoylation sites. The three serine protease active site residues (histidine 75, aspartic acid 215, and serine 220) are all conserved. In addition, the histidine active site LTAAHC (amino acids 71–76) and the serine active site DACQGDSSGGPVA (amino acids 214–225), which represent serine protease signature sequences, are also present in the *B. tropicalis* trypsin sequence [26]. Moreover, most cysteine (5/7) and all histidine (4/4) residues were conserved, as well as aspartic acid 120, which is essential for substrate recognition. The predicted amino acid sequence of the *B. tropicalis* trypsin protein is longer than other group 3 allergens, due to the presence of six additional residues in the proregion.

Conclusions

Molecular analysis of *B. tropicalis* allergens has thus far been limited to the cloning and characterization of five allergens, in contrast to the significant number of *Dermaphagoides* species allergens that have been studied and despite the observation of a significant number of immu-

Fig. 1. Clustal W amino acid sequence alignment of *B. tropicalis* trypsin (Blo t 3) and serine proteases of dust mite origin (Der p 3, Der f 3, Eur m 3). Active site residues are indicated with a ×, and serine and histidine protease signature sites are underlined. Residues which are conserved among the four species are shaded. Arrows indicate conserved cysteine residues. The boxed sequence is a putative tyrosine kinase phosphorylation site. Bold residues indicate significant differences between Blo t 3 and the other sequences.



nodominant proteins in *B. tropicalis* protein extracts. Nucleotide sequence and functional homologies have been demonstrated between *Dermatophagoides* allergens and serine proteases, cysteine proteases, chymotrypsins and amylases, among others [3, 4, 6, 24, 25, 27]. *B. tropicalis* has been shown to express several serine protease activities, including trypsin, elastase, and chymotrypsin [28]. Whether the allergenic potential of proteases results from their biological activity requires further investigations. Cloning and further characterization of allergen genes of mite origin are therefore important in order to fully understand their role in asthma and atopy [29, 30].

We report the cloning and molecular characterization of a novel cDNA from the mite species *B. tropicalis* with high sequence homology to domestic dust mite group 3 allergens, a family of allergens characterized by their trypsin-like protease activity [5]. Screening of a *B. tropicalis* cDNA library resulted in the cloning of a cDNA encoding a 266-amino-acid polypeptide with a deduced molecular mass of 27.5 kD. The *B. tropicalis* trypsin cDNA shares significant amino acid sequence homology with other mite-derived trypsin, namely Der p 3 (49% identity), Der f 3 (48% identity), and Eur m 3 (54% identity). *B. tropicalis* trypsin was also highly homologous at the protein level to trypsin from *Anopheles gambiae* (48% identity), *Drosophila melanogaster* (48% identity), *Homo sapiens* (43% identity), and to a collagenolytic protease (group 9 allergen) from *D. pteronyssinus* (48%).

Sequence comparison of the *B. tropicalis* trypsin and homologous genes from other domestic dust mite species

shows that protease signature sequences and all histidine residues are conserved. Notable differences between *B. tropicalis* trypsin and homologous sequences are C47A and C242R substitutions, the addition of a five amino acid stretch (FYAEG) in the proregion, and a two-residue deletion at the C-terminus (fig. 1). The catalytic activity of serine proteases is mediated by interactions between specific aspartic acid, histidine, and serine residues, linked together by hydrogen bonding, all of which are conserved in the *B. tropicalis* trypsin gene (His75, Asp215, Ser220) [26, 31]. Asp120, which is essential for substrate recognition, was also maintained. Finally, the sequences spanning the serine protease active site's serine (DACQGDSGGPVA) and histidine (LTAHC) are well conserved. The fact that both serine and histidine serine protease active site signatures are conserved strongly supports the inclusion of this novel *B. tropicalis* gene as a new member of the trypsin family of serine proteases.

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