Immunoglobulin E Binding Reactivity of a Recombinant Allergen Homologous to α-Tubulin from Tyrophagus putrescentiae

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Storage mites may cause allergic respiratory diseases in urban areas as well as pose an occupational hazard in rural areas. Characterization of storage mite allergens is important for the development of diagnostic and therapeutic agents against mite-associated allergic disorders. Here we report on the cloning and expression of α-tubulin from the storage mite (Tyrophagus putrescentiae). The deduced amino acid sequence of the α-tubulin from the storage mite showed as much as 97.3% identity to the α-tubulin sequences from other organisms. The highly conserved amino acid sequences of α-tubulins across different species of mites may indicate that cross-reactivity for this potential allergen exists. The frequency of immunoglobulin E reactivity of this recombinant protein is 29.3% in sera from storage mite-allergic subjects.

It is known that sensitization to allergens derived from storage mites may lead to occupational allergic disorders (2, 3). There has been increasing interest in the allergenicity of storage mites in recent years (13). In epidemiological studies, the predominant species in Korean homes were found to be Dermatophagoides farinae (65 to 77%) and Dermatophagoides pteronyssinus (8 to 20%) (7, 15). Tyrophagus putrescentiae was reported to be the third most common domestic mite, infesting 6.5 to 8.5% of Korean homes (7, 15). In a study with sera from atopic urban inhabitants, a marked inhibition of T. putrescentiae-specific immunoglobulin E (IgE) by D. farinae and D. pteronyssinus extract was demonstrated, while D. pteronyssinus- or D. farinae-specific IgE was partially inhibited by a T. putrescentiae extract (14). Significant cross-reactivity between other arthropods and nematodes was also reported (9). The prevalence of storage mite sensitization was reported in a general adult population in Spain (18), and the allergen-specific IgE (CAP) correlation analysis in Spain also showed allergenic similarity between Dermatophagoides spp. and storage mites, such as T. putrescentiae, Glycyphagus domesticus, and Acarus siro (10). Molecular cloning of Tyr p 2 and Tyr p 13 from T. putrescentiae has been done (5, 8). In addition, molecular modeling has found many amino acid substitutions at surface residues of Tyr p 2 that still conserve its tertiary structure (17). These substitutions may indicate that there is limited cross-reactivity for group 2 allergens among different mite species. Moreover, group 13 allergens have not been identified from Dermatophagoides spp. Identification of more allergens and their characterization in molecular detail will allow us to better understand the cross-reactivity for mite species. In this respect, expressed sequence tag (EST) strategies could be useful for the

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identification of cross-reactive allergens present in house dust mite and storage mite (1, 8).

We previously identified several candidate allergen molecules by analyzing an EST database of *T. putrescentiae* (8). Here we describe the expression of recombinant α-tubulin and its allergenic characterization.

An α-tubulin was first identified as a putative allergen from a phage display *Lepidoglyphus destructor* cDNA library (16). There are multiple α-tubulin genes in most organisms, and they are highly conserved within and among species (4, 11, 12).

A cDNA sequence encoding α-tubulin was obtained by analyzing *T. putrescentiae* EST clones. Both strands of the cDNA were sequenced. The insert cDNA was 1,604 bp in length and contained a single open reading frame of 1,364 bp which started at ATG (nt 1) and which terminated at stop codon TAA (nt 1351) (Fig. 1). The estimated molecular mass was 50.04 kDa, and the calculated isoelectric point was 4.834. The deduced amino acid sequence of the *T. putrescentiae* α-tubulin showed as much as 97.3% identity to the α-tubulin sequences from other organisms (Fig. 2). A PROSITE search revealed a tubulin consensus sequence, [SAG]-G-G-T-G-[SA]-G (which is known to interact with GTP at positions 142 to 148), and one N-glycosylation site (positions 380 to 382) (6).

A PCR was carried out to introduce a BamHI site upstream of the start codon and a HindIII site just downstream of the stop codon. The following synthetic oligonucleotides were used as primers: GGGATCCTATGCGAGAATGTATCTC (forward primer) and AAGCTTCCACTTAGAATTCCTCTC (reverse primer) (underlining indicates restriction sites). The resulting PCR fragment was subcloned into the pGEM-T Easy vector (Promega, Madison, WI) and was then digested with BamHI plus HindIII. The DNA fragment was subsequently ligated into the pET 28b vector. When this construct was used, 34 additional amino acids are added at the N terminus of the α-tubulin. The bacterial transformant was induced to express the recombinant protein by the addition of 1 mM isopropyl-1-thio-β-galactopyranoside after the cells had grown to an A_600 of 0.6.

After sonication, a 56-kDa protein was detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the total cell lysate and in the insoluble precipitate. The protein was solubilized in 20 mM Tris, 500 mM NaCl, 5 mM imidazole, and 6 M urea, pH 7.9. It was then purified by using nickel-nitrilotriacetic acid resin (QIAGEN, Valencia, CA) (Fig. 3). The purified protein was desalted and concentrated with a Centriprep filter (YM-10; Millipore, Bedford, MA). The yield of purified recombinant protein was 4.576 mg/liter of bacterial culture.

To test the IgE reactivity of the recombinant protein, an
enzyme-linked immunosorbent assay (ELISA) was performed with sera from *T. putrescentiae*-sensitized subjects diluted 1:4. Sera were obtained from allergic patients attending the Allergy Clinic of the Severance Hospital, Yonsei University, Seoul, Korea; and the diagnosis was based on the patient’s case history and the results of a skin prick test (Allergopharma, Reinbek, Germany). All the subjects were tested for the presence of IgE antibodies against *inbek*, Germay. The numbers on the left are in kilodaltons.

FIG. 3. Purification of recombinant α-tubulin. Proteins were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel and stained with Coomassie brilliant blue. Lanes: M, molecular mass marker; P, pass-through; W1, wash with 5 mM imidazole; W2, wash with 20 mM imidazole; W3, wash with 60 mM imidazole; E, eluate in 1 M imidazole. The numbers on the left are in kilodaltons.

controls plus 2 standard deviations was used as the cutoff value ($P = 0.08$) (Fig. 4).

The IgE reactivity may be influenced by a difference between recombinant and native protein folding or the lack of glycosylation, since no posttranslational modifications occur in the *Escherichia coli* expression system. The recombinant α-tubulin derived from *Lepidoglyphus destructor* showed an 11.6% (11 of 95 samples) IgE-binding frequency (16). Inhibition studies for evaluation of the allergenic potency could not be performed due to its low concentration in the whole-body extract.

Here we elucidated the primary structure of α-tubulin, an important potential allergen, from *T. putrescentiae* and expressed the recombinant protein. The clinical relevance of this allergen remains to be evaluated in vivo. The highly conserved amino acid sequences of α-tubulins among different species may indicate that cross-reactivity for this protein exists. To investigate this possible cross-reactivity, molecular cloning of α-tubulins from house dust mites such as *D. farinae* and *D. pteronyssinus*, which are known to be major sources of mite allergens, is needed. The α-tubulin cloned in this study will facilitate examination of the involvement of this class of allergens in the development of mite-associated allergic diseases. It is also hoped that the use of recombinant α-tubulin in combination with other allergens may improve the diagnostic specificity.

**Nucleotide sequence accession number.** The nucleotide sequence reported in this paper has been deposited in the GenBank sequence database under accession number AY986760.

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AUTHOR’S CORRECTION

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