

Short sequence-paper

Sequencing and high level expression in *Escherichia coli* of the tropomyosin allergen (Der p 10) from *Dermatophagoides pteronyssinus*¹

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Abstract

The cDNA encoding an allergen from the dust mite *Dermatophagoides pteronyssinus* has been cloned and sequenced. The allergen (Der p 10) is a tropomyosin that shared more than 65% identical residues with other invertebrate tropomyosins. The final recovery of recombinant Der p 10 from the culture media after a single purification step was as much as 26 mg/l. The recombinant allergen is reactive to shrimp antitropomyosin IgG antibodies and has a 5.6% frequency of IgE reactivity in sera from mite-allergic patients. © 1998 Elsevier Science B.V.

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IgE-mediated allergy is a serious problem for patients with symptoms such as rhinitis, conjunctivitis, urticaria, angioedema, or asthma. The most significant indoor allergens are those from the two predominant species of the house dust mites, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* [1]. Several allergens from *D. pteronyssinus*, the most common species in Europe, have been studied [2]. Variation in the proportion of major allergens from *D. pteronyssinus* extracts has been reported [3] which could influence their biological

effectiveness in diagnostics and immunotherapy. These variations are due mainly to the growth conditions used in mite cultures and whether total mite cultures or mite bodies are used, because some of these allergens, like Der p 1, are known to be enzymes secreted by the mite during digestion and released in the feces to the culture medium [4]. To overcome these problems, the corresponding cDNA of some mite allergens have been cloned and expressed [2]. Therefore, recombinant techniques will play an important role for investigations on the molecular basis of the IgE response [5,6], and for the development of new diagnostic procedures and possible therapeutic strategies. Recently, Aki et al. [7] cloned and expressed a new major allergen from *D. farinae*, identified as tropomyosin, with an IgE-binding frequency of 80.6% among mite-allergic patients. Tropomyosins are a family of closely related proteins

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¹ The nucleotide sequence reported in this paper has been submitted to the GeneBank™, EMBL and DDBJ Nucleotide Sequences Databases under accession number Y14906.

present in muscle and nonmuscle cells which mediate the interaction between the troponin complex in the actin-linked calcium regulatory systems in striated muscle [8].

cDNA-encoding for *D. pteronyssinus* tropomyosin, called Der p 10 in accordance with the IUIS/WHO Allergen Nomenclature Subcommittee, was obtained by RT-PCR using primers designed according to the nucleotide sequence for *D. farinae* tropomyosin [7]. The PCR fragment was subcloned in pBluescript-II-SK and sequenced using fluorescence-labeled dideoxynucleotides. Sequencing reactions were analyzed on an ABI 373A DNA sequencer (Perkin-Elmer, Foster City, CA). The insert had a length of 855 bp, and encoded a polypeptide of 284 amino acids with a predicted molecular mass of 32.9 kDa and a *pI* of 4.78. High homology (92% identical nucleotides) was found in *D. farinae* tropomyosin (Fig. 1). Only five out of the 65 single-base changes resulted in five amino-acid conversions, the remaining 60 nucleotide substitutions being silent, with a high proportion of pyrimidine transitions (27/60). The absence of an N-X-T/S sequence indicate that there is no N-glycosylation site. The tropomyosin signature, L-K-E-A-E-X-R-A-E, present in all tropomyosins sequenced is also conserved in the C-terminal region of Der p 10. A repeated pattern of seven amino acids constituting the α -helical turn and an extensive leucine zipper motif occurs throughout the sequence as in tropomyosins from other origins. *Dermatophagoides* tropomyosins share 98% identical amino acids. Homology was also very high with tropomyosins from other arthropods such as fruit fly and locust (insects) and shrimps (crustaceans), with 80–75% identity. Homology decreased as phylogenetic distances increased: nematodes (72–69%) and mollusks (65%). Tropomyosins from vertebrates (human, chicken, mouse) contained less than 56% identical amino acids, although the high similarity (74%) could reflect a similar three-dimensional structure. Recent works in shrimp tropomyosins reported six IgE-reactive peptides [9,10] with different degrees of homology to Der p 10. The sequences of the peptides E2, E3, E4, and E6, obtained from *Penaeus aztecus* (brown shrimp) tropomyosin, are highly homologous to Der p 10 (100, 77, 81, and 93%, identity, respectively), while peptide sequences P6 and P, from *P. indicus* (Indian shrimp), have a 65% homology to Der p 10.

M E A I K N K K M Q A M K L E K D N A I D	20
<u>ATGGAGCCCATCAAGAATAAAATGCGAGCAATGAAGTCGAAAGAAATATGCTATCGAT</u>	60
R A E I A E Q K A R D A N L R A E K S E	40
<u>CGAGCTGAAATTCGCCGAACAAAAGCCGTGATGCTAATCTTCCTGCTGAAAGTCCGAA</u>	120
E E V R A L Q K K I Q Q I E N E L D Q V	60
<u>GAAGAAGTACGTGCATTACGAGAAAAATCCCAACAAATCGAGAAATGAATTGATCAGGTT</u>	180
Q E Q L S A A N T K L E E K E K A L Q T	80
<u>CAAGAACAATATTCGGCTGCCAATACAAAATGGAGAAAGGAAAAGCCCTGCAAACT</u>	240
A E G D V A A L N R R I Q L I E E D L E	100
<u>GCTGAAGGTGATGTTGCAGCATTGAATCGTCGTATTCAATTGATCGAAGAAGATTGGAA</u>	300
R S E E R L K I A T A K L E E A S Q S A	120
<u>CGATCAGAAGAACGACTTAAATTCGAACAGCTAAATTCGAAGAAGCATCACAAATTCGCC</u>	360
D E S E R M R K M L E H R S I T D E E R	140
<u>GATGAATCTGAACGTATGCGAAAAATGTTGGAACATCGTTCCTTACCGATGAAGAACGA</u>	420
M E D G L E N Q L K E A R M M A E D A D R	160
<u>ATGGAAGTTTGGAAATCAATTAAGAACACGCTATGATGGCTGAAGATGCTGATAGA</u>	480
K Y D E V A R K L A M V E A D L E R A E	180
<u>AAAATATGATGAAGTTGCACGTAATTTAGCAATGGTTGAAGCCGATTAGACGTCCTGAA</u>	540
E R A E T G E S K I V E L E E E L R V V	200
<u>GAACTGCGGAACTGGTGAATCGAAATTTGTGGAACCTGAGGAAGAAATACGTGTTGTT</u>	600
G N N L K S L E V S E E K A Q Q R E A	220
<u>GGTAATATCTAAATCATTTGGAAGTTAGTGAAGAAAAAGCACAACGCTGAAGAAGCA</u>	660
H Y E Q Q I R I M T T A K L K E A E A R A E	240
<u>CATGAACAACAGATCCGTATTATGACACAACTTAAAGAAAGTGAAGACGCTGCTGAA</u>	720
F A E R S V Q K L Q K E V G D R L E D E L	260
<u>TTGCTGAACGATCAGTGCAGAAATTAACAAAAGAGTTGGTGGTTGGAAAGATGAATTG</u>	780
V H E K E K Y K S I S D E L D Q T F A E	280
<u>GTCCATGAAAGGAAAAATATAATCCATTTCGGATGAATTGGACCAACATTTGCCGAA</u>	840
L T G Y *	285
<u>CTTACTGGTTATTA</u>	855

Fig. 1. Complete nucleotide and amino acid deduced (in bold) sequences of Der p 10. Differences with Der f 10 [7] are shown with the corresponding amino-acid change when it occurs. Primers used for cDNA amplification are underlined in the nucleotide sequence. IgE-reacting epitopes, defined by peptides P6 and P9 [9] and peptides E2, E3, E4, and E6 [10], are marked up and down of the Der p 10 amino-acid sequence, respectively.

The significant homology of Der p 10 to other invertebrate tropomyosins and to the described IgE-binding epitopes could explain the high cross-reactivity found between arthropods (insects and crustaceans) and mollusks (gastropods and cephalopods) [11–13], and supports the idea that tropomyosin might represent a pan allergen [14].

A nonfusion form of Der p 10 was expressed in *E. coli* BL21 (DE3) [15], using the expression vector pKN172 [16] as described previously [17]. The yield of recombinant Der p 10 (rDer p 10) was 43 mg/l of bacterial culture, which represented approximately 15% of total *E. coli* proteins. The recombinant pro-

tein was purified by isoelectric precipitation [7] and gel filtration chromatography in a Superdex 75 PC 3.2/30 column (SMART, Pharmacia Biotech). The isoelectric precipitation method gave a 96%-pure preparation, resulting in a band with an apparent molecular mass of 36 kDa, as determined by SDS-PAGE (Fig. 2A), which is in good agreement with the deduced molecular mass from the nucleotide sequence. Repeated extractions of the first precipitate were necessary for improving rDer p 10 recovery. The yield of purified rDer p 10 was 26 mg/l of bacterial culture. For comparison, the yield of the other recombinant mite tropomyosin (Der f 10) [7] was 79-fold lower and had the disadvantage of being fused to glutathione S-transferase, a protein known to be also a mite allergen [18]. The residual contaminating band present at 48 kDa could be removed by size-exclusion chromatography on a Superdex 75 column, giving a preparation of more than 99% purity. In the presence of 6 M urea, the mobility of the purified rDer p 10 on SDS-PAGE changed to give an apparent molecular mass of 48 kDa, whereas control proteins migrated at the expected positions (Fig. 2B). The isoelectric behavior and the change in electrophoresis migration in urea gels are characteristics of the tropomyosins [8] and indicate that rDer p 10 expressed in *E. coli* maintained physicochemical properties of natural tropomyosins. The reactivity of

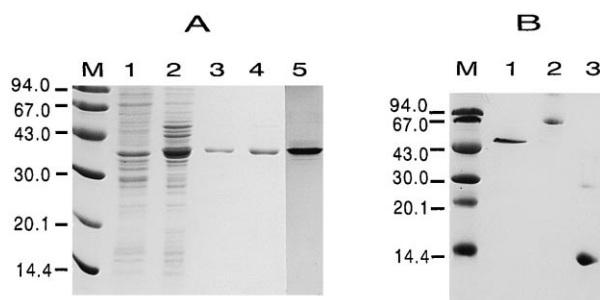


Fig. 2. Expression and purification of rDer p 10 expressed in *E. coli*. (A) 12.5% SDS-PAGE, lanes 1–4 were Coomassie Blue-stained and lane 5 was silver-stained. Lane M, molecular mass markers; lanes 1 and 2, total cell lysate from *E. coli* BL21 (DE3) containing pKN172 alone and pKN172 with tropomyosin-encoding cDNA, respectively; lanes 3 and 4, rDer p 10 after isoelectric precipitation (3 and 6 μ g, respectively); lane 5, rDer p 10 after size exclusion chromatography; (B) 12.5% SDS-PAGE containing 6 M urea. Lane 1, rDer p 10; lane 2, BSA; and lane 3, cytochrome c.

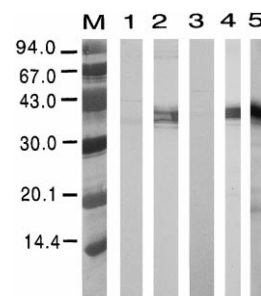


Fig. 3. Western blotting of rDer p 10 probed with: commercial chicken tropomyosin antiserum (Sigma Chemicals, St. Louis, MO) (lane 1); shrimp tropomyosin antiserum, experimentally obtained after immunization of white New Zealand rabbits with purified shrimp tropomyosin (lane 2); and serum pool from healthy individuals (lane 3); serum from a monosensitized dust-mite-allergic patient (lane 4); and serum from a dust-mite-allergic patient also sensitized to household insects (lane 5).

rDer p 10 to antibodies against shrimp and chicken tropomyosins (Fig. 3) is a consequence of the different levels of amino-acid homology, 80% for shrimp and 55% for chicken tropomyosins.

Western blotting [19] of rDer p 10 probed with sera from monosensitized dust-mite-allergic patients showed that this recombinant allergen reacted with specific IgE in only 4 of 71 allergic patients (5.6%). This result contrasts with the high incidence (80.6%) of Der f 10 reported by Aki et al. [7] in a population of 31 dust-mite-allergic patients. Polysensitization of this population to other invertebrates (which is not reported in the above-mentioned article) could be responsible for this incidence, considering the high cross-reactivity found between mite and other invertebrates such as crustaceans, mollusks, cockroaches, and chironomids [11,20–22]. Preliminary results in our lab pointed in this direction, because a IgE-binding reactivity to mite tropomyosin was found in sera from mite-allergic patients polysensitized to household insects (Fig. 3).

In conclusion, results reported here show that this heterologous expression system and purification procedure are suitable for the production, in high amounts, of well-characterized and purified rDer p 10, which could allow more detailed immunological characterization of tropomyosins as allergens.

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