

Molecular Cloning and the Allergenic Characterization of Tropomyosin from *Tyrophagus putrescentiae*

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Abstract: Storage mites have been recognized as a cause of asthma and rhinitis. Studies from several countries have shown that the IgE-mediated allergy to storage mites is of considerable importance, especially in rural populations. This study aimed to identify and characterize new allergens from *Tyrophagus putrescentiae*. A partial cDNA sequence encoding tropomyosin was isolated from the cDNA library by immunoscreening using anti-mouse IgG1 sera raised against *T. putrescentiae* whole body extract. The deduced amino acid sequence shares 64-94% identity with previously known allergenic tropomyosins. Its recombinant protein was produced by using a pET 28b expression system and purified by affinity chromatography using Ni-NTA agarose. The IgE reactivities of tropomyosins from *T. putrescentiae* and *Dermatophagoides farinae* were compared by enzyme linked immunosorbent assay (ELISA). Recombinant Tyr p 10 showed 12.5% (5/40) IgE-binding reactivity, whereas recombinant Der f 10 showed 25% (10/40) IgE-binding reactivity against the same sera from storage mite-sensitized and house dust mite-sensitized subjects. Both recombinant Tyr p 10 and Der f 10 showed little inhibition of IgE binding to *T. putrescentiae* crude extract by ELISA. Tropomyosin seems to contribute only a small portion of the cross-reactivity with house dust mites.

Keywords: Allergen, cross-reactivity, tropomyosin, *Tyrophagus putrescentiae*.

INTRODUCTION

Storage mites usually infest stored food products and decrease their safety by producing potent allergens which can cause occupational disorders [1, 2]. Allergens derived from storage mites are demonstrated to be a major sensitizing agent even in patients without occupational exposure in Spain [3]. Sensitization to storage mite allergens has often been demonstrated not only in rural areas but also in urban areas [4, 5]. It is controversial, however, whether or not the allergic response to storage mites is triggered by cross-reactive allergens from house dust mites.

Tropomyosin, a mite group 10 allergen, could be a suitable molecule for the investigation of storage mite sensitization in this case, because it has been known to be highly cross-reactive [6-8]. *Tyrophagus putrescentiae* was found to be a dominant species of storage mites in Korea [9, 10]. Previously, we identified potential allergens homologous to known house dust mite allergens by the analysis of expressed sequence tags [11], and the allergenicities of candidate allergens are now being investigated. At the same time, we isolated a partial cDNA fragment encoding tropomyosin by the immunoscreening of the *T. putrescentiae* cDNA library using mouse anti-*T. putrescentiae* sera. The full sequence was obtained by reverse transcriptase-PCR (RT-PCR) using a degenerate primer designed on the basis of known allergenic

tropomyosins (TrpmF1) [12] and gene-specific primers designed on the internal sequence of the cloned cDNA fragment (Tp10R1 and Tp10R2).

We also cloned and expressed recombinant tropomyosin from *Dermatophagoides farinae*, which is the dominant species in Korea, and compared its allergenicity with *T. putrescentiae* tropomyosin, because it is reported to be highly allergenic [13]. The IgE-binding reactivity of recombinant tropomyosin was examined by ELISA.

MATERIALS AND METHODS

Subjects and Serum Samples

Sera were obtained from 40 patients (age range, 7 to 69 years; average 32 years) who attended the Allergy Clinic in Yonsei University College of Medicine, Seoul, Korea. The diagnosis of allergic diseases was based on case history and a skin prick test. Diagnosis followed the revised nomenclature [14]. All sera were tested for the presence of IgE antibodies to *T. putrescentiae* using the Uni-CAP system and ELISA. The serum samples (n = 18; age range, 17 to 76; average 49 years) obtained from subjects who showed a negative reaction both to the skin prick test and ELISA were used for the negative control.

Immunization of the Mice

One, five, and 10 µg of crude extract in alum adjuvant were immunized to three mice each. One mouse was injected with PBS as a negative control. Mice were further challenged at day 21, 42, and 63, and the sera were collected on the days of challenge. The titer of specific IgG1 was evalu-

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ated by ELISA. Mice injected with one μg showed the highest titer and their pooled serum was used for the immunoscreening of the cDNA library.

The Construction of the *T. putrescentiae* cDNA Library

The frozen sample of *T. putrescentiae*, which was mass-reared in our laboratory [15], was homogenized in liquid nitrogen. The mRNA was purified by using the mRNA isolation kit (Stratagene, La Jolla, CA). The cDNA library was constructed using a ZAP Express cDNA Gigapack II gold cloning kit (Stratagene) according to the manufacturer's instructions.

Screening of the cDNA Library

Screening of the *T. putrescentiae* cDNA library was carried out by plaque immunoassay using a *picoBlue* immuno-screening kit (Stratagene). A 1:500 diluted pooled serum from mice immunized with the crude extract of *T. putrescentiae* and alkaline phosphatase-labeled anti-mouse IgG1 was used for the immunoassay. Ten positive plaques were selected and further purified to homogeneity. The phagemid was excised *in vivo* with the help of an ϕ 1 helper phage as described in the instruction manual of the Zap-cDNA synthesis kit (Stratagene). DNA was isolated and subjected to sequencing using the ABI PRISM Dye Terminator Cycle sequencing Ready Kit (Perkin-Elmer Applied Biosystems, Foster City, CA.). The sequence alignment and homology analysis were performed using the BLAST network service provided by the National Center for Biotechnology Information (NCBI) and the CLUSTAL X program (<ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX>) [16]. Two of 10 selected clones showed high homology with tropomyosin.

The Molecular Cloning of Tropomyosin by Reverse Transcriptase-Polymerase Chain Reaction

To obtain information on the 5'-region of tropomyosin, RT-PCR was performed using a degenerate primer, TrpmF1, (forward primer, GGA(C/G/T)GC(C/G/T)ATCAAGAA(A/G/T)AA(A/G)A(C/T)T), which was designed on the basis of known allergenic tropomyosins [12] and *T. putrescentiae*-specific primer, Tp10R1, (reverse primer, GCAGGTGTGTTTGCTTGTCG). Nested PCR was carried out with TrpmF1 and the *T. putrescentiae*-specific primer, Tp10R2, (GATGCAGTAGCCGGTGAG). The total RNA samples of both *T. putrescentiae* and *D. farinae* were extracted using the TRizol reagent (Invitrogen, Carlsbad, CA). To synthesize the cDNA, reverse transcription was initiated with 5 μg of the total RNA for each reaction. After 5 minutes of initial denaturation at 95°C, 35 cycles of reaction (each consisting of 30 seconds at 94°C, 30 seconds at 53°C, and one minute at 72°C) were performed. These cycles were followed by a final extension at 72°C for 9 minutes. The PCR-amplified fragment was isolated from 1% agarose gel and ligated into pGEM-T Easy vector (Promega, Madison, WI) before sequence determination.

The Expression and Purification of Recombinant Tropomyosin

The open reading frames of the tropomyosins from *T. putrescentiae* and *D. farinae* were amplified by RT-PCR as

described above. The primers used were: Tp10F (forward primer, ATGGACGCTATCAAGAATAA), Tp10R (reverse primer, GGTAGATGCCTAGTAGCCGG), Df10F (forward primer, ATGGAGGCCATCAAGAAA), and Df10R (reverse primer, CTGTCTGCGTAATATGAAAG). They were cloned into the pGEM-T Easy vector and then subcloned into the *EcoR* I-cut site of the pET 28b expression vector (Novagen, Madison, WI). The recombinant tropomyosins were over-expressed in *Escherichia coli* BL21 (DE3). The cells were grown at 37°C in Luria Bertani broth at an absorbance of 600 nm of 0.5 containing 50 mg/ml of kanamycin. Then, isopropyl-1-thio- β -galactopyranoside (1 mM) was added to the culture to induce the expression of recombinant proteins. Cells were harvested by centrifugation after being induced for four hours, and the pellets were resuspended in lysis buffer (10 mM imidazole, 300 mM NaCl, 50 mM NaH_2PO_4 , pH 8.0), and lysed using a French press. Recombinant tropomyosins were purified using the Ni-NTA-agarose (Qiagen, Valencia, CA) according to the manufacturer's instructions under native conditions. Purified proteins were analyzed using a 10% polyacrylamide gel containing sodium dodecyl sulfate [17].

Specific IgE-Binding to Recombinant Tropomyosins

The reactivities of the specific IgE antibodies to recombinant tropomyosin were examined by an ELISA. In brief, each well of microtiter plates was coated with the recombinant tropomyosins (200 ng/well) in a 0.05 M sodium bicarbonate buffer at pH 9.6. Plates were blocked with 3% skim milk in PBS containing 0.05% Tween 20 (PBST). The plates were then incubated for one hour with 50 μl /well of 1:4 sera diluted in PBST containing 1% BSA. IgE antibodies were detected using biotinylated goat anti-human IgE (epsilon chain specific; vector, Burlingame, CA) and streptavidin-peroxidase (Sigma). Absorbance was measured by using an automatic microplate reader (TECAN, Salzburg, Austria). The assay was carried out in duplicate. The mean absorbance level of the serum samples from 18 healthy controls plus two standard deviations was used as the cut-off value. For the inhibition test, 100 μl of 10 $\mu\text{g}/\text{ml}$ of *T. putrescentiae* extract was coated overnight at 4°C. The 1:2 diluted serum was pre-incubated with various concentrations of recombinant proteins or crude extracts overnight at 4°C. Subsequently, IgE antibodies were detected as described above.

RESULTS

The DNA and Protein Sequence Analysis of *T. putrescentiae* Tropomyosin

The partial fragments of cDNA encoding tropomyosin were isolated by three rounds of immunoscreening. The fragments were truncated only 12 nucleotides long at the 5'-region of their open reading frame. The amino acid sequence homology search revealed that *T. putrescentiae* tropomyosin shared a high homology with other invertebrate tropomyosins. The cDNA sequence coding for the N-terminal region was cloned by RT-PCR using degenerated (forward) primer and gene specific (reverse) primer. The cloned sequence encoded 284 amino acids with an estimated molecular mass of 32,956 Da and an isoelectric point of 4.60 (Fig. 1). *T. putrescentiae* tropomyosin shared 64-94% of its amino acids se-

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atggacgtatcaagaataagatgcaggcgatgaagctcaggaggacaatgccatcgac 60
M D A I K N K M Q A M K L E E D N A I D
cgagctgagattgccgagcagaaggctcgatgccaaactcaagtctgagaagactgag 120
R A E I A E Q K A R D A N L K S E K T E
gaggaagttcgcgtctcagaagaagatccagcagattgagaacgaactggaccaagtc 180
E E V R A L Q K K I Q Q I E N E L D Q V
caggagaacctgacccaggccaccaccaagctcaggagaaggagaaggccctccagact 240
Q E N L T Q A T T K L E E K E K A L Q T
gccgaagccgatgtcgtcgcctcaaccgacgcacatcgatcaggaggacctcgag 300
A E A D V A A L N R R I Q L I E E D L E
cgatctgaggagcgtctcaaggtggccaccgcaagcttgaggaggctccattccgt 360
R S E E R L K V A T A K L E E A S H S A
gatgaatccgagcgcgcgaagatgcttgaccacgaagcatcaccgatgaggagcgc 420
D E S E R M R K M L E H R S I T D E E R
atggacggtcggagagccagctgaaggaggccgcctgatggcaggagcgtgacgcc 480
M D G L E S Q L K E A R L M A E D A D R
aagtcgatgaggtgcgcgaagctggccatggtgaagctgatctggagcgcgtgag 540
K Y D E V A R K L A M V E A D L E R A E
gagcgtgccgagactggtgaatcaagatcgctgagctcaggaggagcgtcgcgtcgtc 600
E R A E T G E S K I V E L E E L R V V
ggcaacaacctgaagctctcgaagtcagcaggagaaggcccaacaacgcgaggaggcc 660
G N N L K S L E V S E E K A Q Q R E E A
tatgagcaacaatccgcacatgactctaaagctcaaggaggctgaggtcgcgcgcgag 720
Y E Q Q I R I M T S K L K E A E A R A E
tttgctgagcgtatcggttcagaagctcagaaggaggtcgaccgactggaagacgagctg 780
F A E R S V Q K L Q K E V D R L E D E L
gtgcacgagaaggaaaagtagcagatcaatctcgacgagctggaccagaccttgcgcgag 840
V H E K E K Y E S I S D E L D Q T F A E
Ctcaccggtactaggcatccaatcactagtg 875
L T G Y

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Figure 1. The nucleotide and deduced amino acid sequences of Tyr p 10. This sequence was deposited in GenBank under accession number AY623832.

quence identity with previously reported allergenic tropomyosins (Fig. 2). *T. putrescentiae* tropomyosin was designated as Tyr p 10 according to the guidelines of the International Union of Immunological Societies Allergen Nomenclature Subcommittee [18] (Fig. 1).

The cDNA fragment encoding *D. farinae* tropomyosin (Der f 10) was also cloned by RT-PCR for comparison. The cDNA sequence cloned in this study showed differences at three positions: position 99 (T to C), 297 (G to A), and 318 (T to C). Its conceptually translated amino acid sequence, however, was identical with that of the previously reported Der f 10 (GenBank accession number D17682).

The Expression and Purification of Recombinant Tropomyosins

Both Tyr p 10 and Der f 10 were subcloned into the pET-28b expression vector. The recombinant proteins produced bands of 42 kDa when determined by SDS-PAGE while their calculated molecular masses (including their histidine tagging proteins at the N-terminus) were 36,883 Da, both for Der f 10 and Tyr p 10 (Fig. 3). The yields of purified proteins were 4.745 mg/l of bacteria culture for recombinant Tyr p 10 and 3.781 mg/l for recombinant Der f 10 as measured by a Bradford assay (Bio-Rad, Hercules, CA).

The IgE-Binding of Recombinant Proteins

The prevalence of specific IgE against recombinant Tyr p 10 and Der f 10 were examined by ELISA. Twelve sera (24%) could be considered to be positive for recombinant Tyr p 10 or Der f 10 (Fig. 4). Five of 40 sera (12.5%) tested were shown to be positive for recombinant Tyr p 10 and 10 of 40 (25%) for recombinant Der f 10 (Fig. 4). Among them three sera were positive for both recombinant proteins. Three showed stronger reactions to recombinant Tyr p 10, and three showed stronger reactions to recombinant Der f 10. Four sera showed positive reactions to both recombinant Tyr p 10 and Der f 10.

The Inhibition of Specific IgE Binding to Crude Extracts

An inhibition study was performed to investigate the relative potency of the tropomyosin in *T. putrescentiae* extract using sera from the subject who showed the strongest reaction to recombinant Tyr p 10 and from the subject who showed the strongest reaction to recombinant Der f 10. Recombinant Tyr p 10 and Der f 10 inhibited IgE binding to the *T. putrescentiae* extract at a rate of less than 20% at a concentration of 1-10,000 ng/ml of inhibitor, whereas *T. putrescentiae* extract showed a maximum inhibition rate of 98.3% at an inhibitor concentration of 10 µg/ml (Fig. 5).

DISCUSSION

Allergen specific immunotherapy has mainly been used in the treatment of allergic rhinitis and insect venom anaphylaxis [19]. Crude allergen extract, however, may cause side effects such as anaphylaxis. Immunotherapeutics, using modified recombinant allergens with reduced allergenicity and increased antigenicity, are believed to be the most promising approach for the treatment of allergic disorders [20]. In the context of immunotherapeutics, it is important to characterize individual allergens and to analyze IgE-binding epitopes.

By immunoscreening the cDNA library of *T. putrescentiae*, we were able to isolate the cDNA encoding tropomyosin (Fig. 1) and showed that its deduced amino acid sequence was highly conserved compared with allergenic tropomyosins from other sources (Fig. 2). We have produced the recombinant Der f 10 for comparison with recombinant Tyr p 10, since Aki *et al.*, has stated that it showed a high IgE-binding frequency (80.6%) among the sera of patients who are allergic to house dust mites (1995) [13].

In Fig. 4, only four of 12 sera showed positive reactions to both recombinant Tyr p 10 and recombinant Der f 10. In addition, three of the sera showed stronger IgE-binding reactivity to recombinant Tyr p 10 than to recombinant Der f 10. These results may imply that the IgE-binding epitopes are distributed in various regions. The low degree of inhibition of IgE binding to *T. putrescentiae* extract by recombinant Tyr p 10 and Der f 10 may imply that there is only a minor degree of cross-reactivity between house dust mite and storage mite antigens and distinct IgE binding epitopes between them. Minimal inhibition between recombinant Tyr p 10 and recombinant Der f 10 supports this observation (data not shown), even though considerable cross-reactivity between

	10	20	30	40	50	60	70	80	90	100	
Tyr p 10	MDATKNKQAMKLEEDNAIDRAETAEQKARDANKSEKTEEEVRALQKKIQIENELDQVQENLTQATTLKEEKALQTAADVAALNRRIQLIEEDLE										100
Lep d 10	E.....K.....S.....RA.....S.....G.....S.....N.....S.....G.....										100
Der f 10	E.....K.....K.....RA.....S.....Q.....SA.....N.....G.....										100
Der p 10	E.....K.....K.....RA.....S.....Q.....SA.....N.....G.....										100
Lep s 1	E.....K.....K.....M.....K.....DAL.....AQ.....R.....AD.....IL.....QD.....K.....PS.....V.....TDFTTK.....AT.....NKN.....D.....T.....TNT.....SE.....S.....KV.....M.....N.....										100
Per a 7	K.....K.....M.....LLC.....Q.....RA.....A.....A.....S.....D.....TN.....Q.....M.....YNA.....D.....D.....N.....SE.....L.....										100
Per f 7	K.....K.....M.....LLC.....Q.....RA.....A.....A.....S.....D.....TN.....Q.....M.....YNA.....D.....D.....N.....SE.....L.....										100
Bla g 7	K.....K.....M.....LLC.....Q.....IRA.....A.....A.....S.....D.....TN.....Q.....M.....YNA.....D.....D.....N.....SE.....L.....										100
Chi k 10	K.....K.....L.....LLC.....NQ.....RA.....A.....A.....T.....T.....D.....T.....GQE.....LYNG.....G.....N.....SE.....LG.....D.....										100
Met e 1	K.....M.....DTL.....QNKE.....NRA.....S.....HN.....RM.....L.....D.....S.....LK.....NQ.....V.....D.....SN.....GE.....L.....										90
Hom a 1	K.....K.....M.....DTL.....QNKE.....IRA.....I.....ITH.....M.....V.....Q.....SL.....N.....N.....GE.....L.....										100
Cha f 1	K.....K.....M.....DTL.....QNKE.....RA.....I.....T.....M.....V.....A.....Q.....SA.....N.....D.....N.....GE.....P.....										100
Pen a 1	K.....K.....M.....DTL.....QNKE.....NRA.....S.....HN.....RM.....L.....D.....S.....LK.....NQ.....V.....D.....SN.....GE.....L.....										100
Pan s 1	K.....M.....DTL.....QNKE.....IRA.....A.....HN.....RM.....L.....D.....S.....LK.....NQ.....V.....D.....SN.....GE.....L.....										90
Pen i 1	K.....YDK.....G.....LE.....LVC.....E.....TRA.....S.....A.....HE.....RM.....L.....D.....S.....LK.....NQ.....V.....D.....SN.....GE.....L.....										100
Tur c 1	AANL.....DF.....N.....N.....Q.....QD.....LSK.....V.....D.....SN.....GE.....T.....L.....									51	
Cra g 1	NSARGF.....T.....N.....KYQEQ.....N.....A.....TASE.....GEIQS.....									49	
Ani s 3	K.....I.....K.....L.....DA.....E.....V.....QMTD.....L.....RI.....L.....DT.....WM.....T.....D.....KA.....D.....ST.....NSN.....KV.....E.....E.....MT.....L.....E.....										100

	110	120	130	140	150	160	170	180	190	200	
Tyr p 10	RSEERLKVATAKLEASHSADESENRKILHRSTTDEERNDGLSEQLKEARLMAEDADRYKDEVARKLANVEADLERAEERAEETGESKIVELEELRVV										200
Lep d 10	G.....I.....S.....Q.....E.....M.....										200
Der f 10	I.....Q.....N.....M.....										200
Der p 10	I.....Q.....N.....M.....										200
Lep s 1	GT.....LT.....G.....A.....AS.....C.....V.....N.....QQ.....Q.....TN.....M.....G.....S.....S.....M.....Q.....D.....V.....D.....VKS.....D.....M.....K.....										200
Per a 7	AT.....A.....QA.....A.....I.....SKGLA.....A.....N.....F.....E.....K.....S.....										200
Per f 7	AT.....A.....QA.....A.....I.....SKGLA.....A.....N.....F.....E.....K.....S.....										200
Bla g 7	AT.....A.....QA.....A.....I.....SKGLA.....A.....N.....F.....E.....K.....S.....										200
Chi k 10	AS.....S.....AA.....A.....I.....N.....LA.....A.....N.....FL.....E.....K.....A.....A.....										200
Met e 1	NT.....T.....A.....QA.....V.....N.....LS.....A.....N.....FL.....E.....										190
Hom a 1	NT.....T.....A.....QA.....V.....N.....LS.....A.....N.....FL.....E.....										200
Cha f 1	NT.....T.....A.....QA.....V.....N.....LS.....A.....N.....FL.....E.....S.....										200
Pen a 1	NT.....T.....A.....QA.....V.....N.....LS.....A.....N.....FL.....E.....										200
Pan s 1	NT.....T.....A.....QA.....V.....N.....LS.....A.....N.....FL.....E.....F.....										190
Pen i 1	GS.....A.....QA.....A.....I.....N.....ALA.....A.....N.....FL.....E.....										200
Tur c 1	N.....QT.....ER.....KA.....V.....N.....LS.....A.....N.....FYI.....ED.....E.....IT.....V.....A.....L.....AA.....A.....VL.....K.....										151
Cra g 1	NT.....T.....A.....QA.....V.....N.....LS.....A.....N.....FL.....E.....										149
Ani s 3	A.....L.....T.....T.....V.....VN.....N.....FQ.....ANTV.....QML.....E.....T.....N.....										200

	210	220	230	240	250	260	270	280	
Tyr p 10	GNNLSLEVSEKAAQREAEYEQIRIMTSKLEAEARAEFAERSYQKQKENDRIEDELVHEKEKYESISDELQDTFNAELTGY-								284 [100 %]
Lep d 10	K.....								284 [93.3%]
Der f 10	K.....								284 [94.0%]
Der p 10	H.....T.....G.....VHE.....								283 [92.2%]
Lep s 1	S.....N.....V.....E.....KR.....KTL.....V.....Y.....KY.....K.....GJN.....DR.....RALA.....M.....S.....								284 [64.0%]
Per a 7	M.....E.....K.....KTL.....TR.....								284 [78.5%]
Per f 7	M.....E.....K.....KTL.....TR.....								284 [78.5%]
Bla g 7	M.....E.....K.....KTL.....TR.....								284 [78.1%]
Chi k 10	N.....E.....KN.....KTL.....TR.....								285 [76.1%]
Met e 1	N.....KE.....KTL.....N.....A.....								274 [78.1%]
Hom a 1	N.....KE.....KTL.....N.....A.....								284 [80.2%]
Cha f 1	N.....T.....KE.....KTL.....N.....A.....								264 [79.9%]
Pen a 1	N.....KE.....KTL.....N.....A.....								284 [78.5%]
Pan s 1	N.....KE.....KTL.....N.....A.....								274 [78.4%]
Pen i 1	SN.....E.....KN.....KTL.....TR.....								285 [74.2%]
Tur c 1	I.....Q.....S.....DS.....ET.....DL.....QR.....A.....L.....S.....LA.....KA.....A.....								235 [71.0%]
Cra g 1	M.....I.....QE.....S.....DS.....ET.....DL.....QR.....D.....N.....TE.....T.....S.....LA.....R.....KA.....A.....								233 [63.5%]
Ani s 3	L.....DS.....E.....TVSAR.....T.....								284 [74.2%]

Figure 2. The comparison of Tyr p 10 with different tropomyosin: Tyr p 10 (*Tyropagus putrescentiae*), Lep d 10 (*Lepidoglyphus destructor*, Q9NFZ4), Der f 10 (*Dermatophagoides farinae*, baa04557), Der p 10 (*Dermatophagoides pteronyssinus*, O18416), Lep s 1 (*Lepisma saccharina*, AJ309202), Per a 7 (*Periplaneta americana*, AAD19606), Per f 7 (*Periplaneta fuliginosa*, Q8T6L5), Bla g 7 (*Blattella germanica*, Q9NG56), Chi k 10 (*Chironomus kiiensis*), Met e 1 (*Metapenaeus ensis*, Q25456), Hom a 1 (*Homarus americanus*, O44119), Cha f 1 (*Charybdis ferialis*, Q9N2R3), Pen a 1 (*Penaeus aztecus*, DQ151457), Pan s 1 (*Panulirus stimpsoni*, O61379), Pen i 1 (*Penaeus indicus*, Shanti et al., 1993), Tur c 1 (*Turbo cornutus*, Ishikawa et al., 1998), Cra g 1 (*Crassostrea gigas*, AF239173), Ani s 3 (*Anisakis simplex*). The amino acid sequences of Pen i 1 and Tur c 1 were obtained directly from the articles. The percentage of each sequence identity was given in the parentheses. The shaded areas indicate the IgE-binding regions.

T. putrescentiae and *Dermatophagoides* species has been described [21].

A number of IgE-binding epitopes have been described from shellfish (Fig. 2). Two IgE-binding regions (amino acid residues 50-66 and 153-161) for tropomyosin in the Indian shrimp, *Penaeus indicus*, (Pen i 1) were identified by the analysis of nine trypsin fragments [22]. Four IgE-binding regions (E2, 167-179; E3, 136-148; E4, 262-282; E6, 153-161) for tropomyosin in the brown shrimp, *Penaeus aztecus*, (Pen a 1) were reported by analyzing a recombinant Pen a 1

peptide library [23]. In addition, five IgE-binding regions (region 1, 43-57; region 2, 85-105; region 3, 133-148; region 4, 187-202; region 5, 247-284) were identified by the analysis of 46 synthetic, overlapping peptides [7]. Maleylated tropomyosin from the gastropod, *Turbo cornutus* (Tur c 1) was digested with trypsin and four tryptic digests (MT 15, 16, 17 and 18) corresponding to the C-terminal region (residues 245-284) of Tur c 1, and was reported as one of the IgE-binding epitopes [24]. Substantial inhibition of IgE antibodies from crustacean and mollusk allergic sera to Tur c 1 has been shown. The lysylendopeptidase and trypsin digested

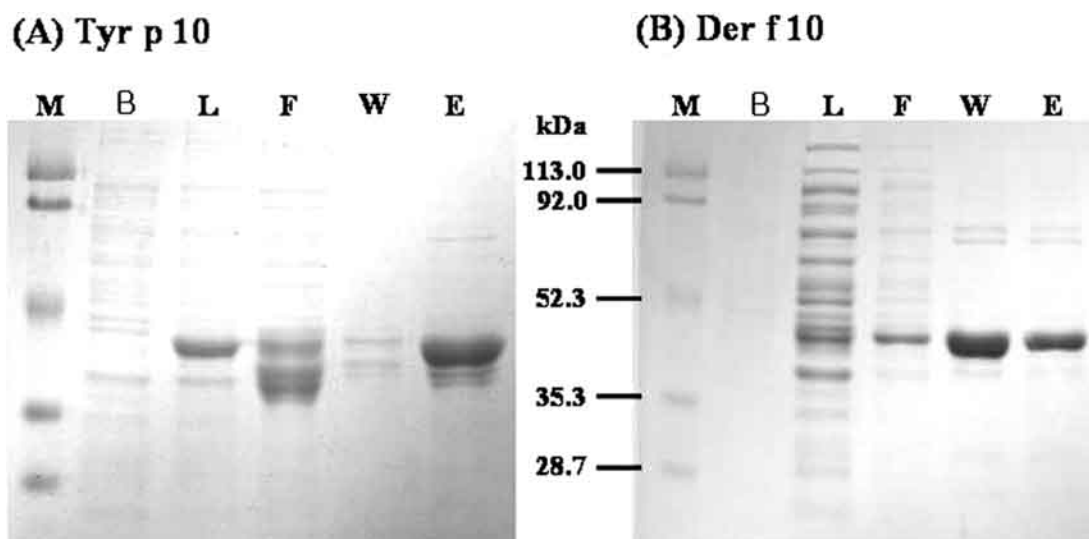


Figure 3. The expression and purification of recombinant Tyr p 10 (A) and Der f 10 (B). Proteins were separated on 10% acrylamide gel and stained with Coomassie brilliant blue. Lanes: M: molecular mass marker; B: total lysate from uninduced cells; L: total cell lysate bearing recombinant protein after IPTG; F: fraction flowed through the column; W: fraction washed the column; E: fraction eluted.

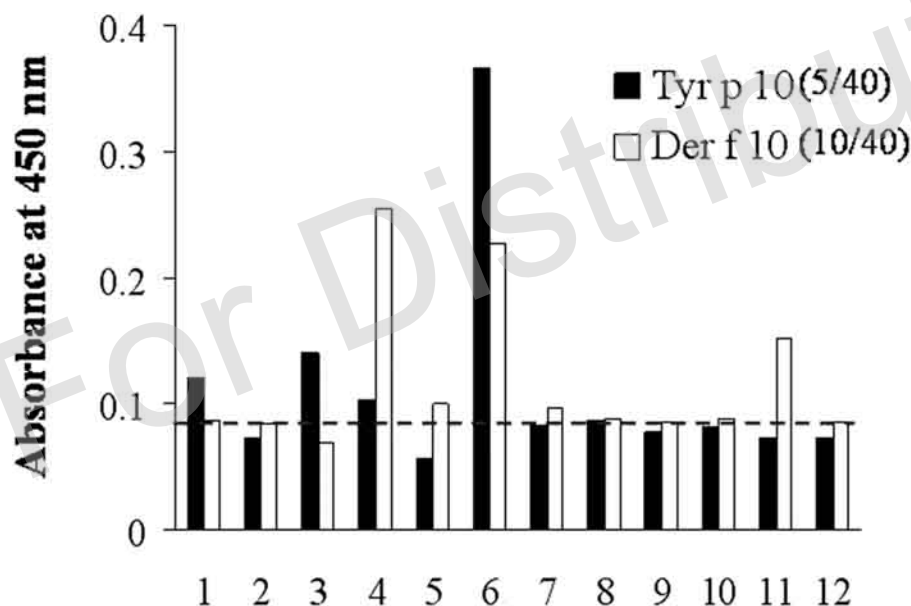


Figure 4. The IgE reactivity of human sera against recombinant tropomyosins. The dotted line indicates the cut-off value.

peptides of tropomyosin from the Pacific oyster, *Crassostrea gigas* (Cra g 1) were generated and their inhibitory capacity for IgE-binding of the sera from crustacean and mollusk allergic patients was examined by ELISA [25]. The peptide K21 (92-105) was reported to retain the most inhibitory capacity for IgE-binding.

The IgE-binding reactivity of five recombinant fragments of tropomyosin from *Blattella germanica* (Bla g 7) was investigated, and multiple IgE-binding epitope regions were demonstrated [26]. Ayuso *et al.*, have also reported that the five IgE-binding epitopes are positioned at regular intervals [7]. These results may suggest a relationship with the repetitive coiled-coil structure of tropomyosin [27]. Ayuso *et al.*, have characterized the IgE-binding epitopes in Pen a 1 that cross-react with other allergenic tropomyosins in house dust

mites and cockroach [7]. They suggested the molecular basis of cross-reactivity among invertebrate species based on the amino acid sequence similarity and epitope reactivity. It has recently been described, however, that the injection of house dust mite extracts for allergen specific immunotherapy does not seem to induce *de novo* tropomyosin sensitization in mite allergic patients [28]. We found many amino acid variations in the regions which have been known as IgE-binding epitopes among other species (Fig. 2). These reports may suggest that there are not only common IgE-binding epitopes but also unique epitopes for each tropomyosin. Unique IgE-binding epitopes have been described between the tropomyosins of *Blomia tropicalis* (Blo t 10) and *Dermatophagoides pteronyssinus* (Der p 10), even though they are highly conserved (95% amino acids identity) [29]. Further studies on the structure, conformation, and physiology of T cell epi-

topes as well as B cell epitopes are necessary for immunotherapeutic applications.

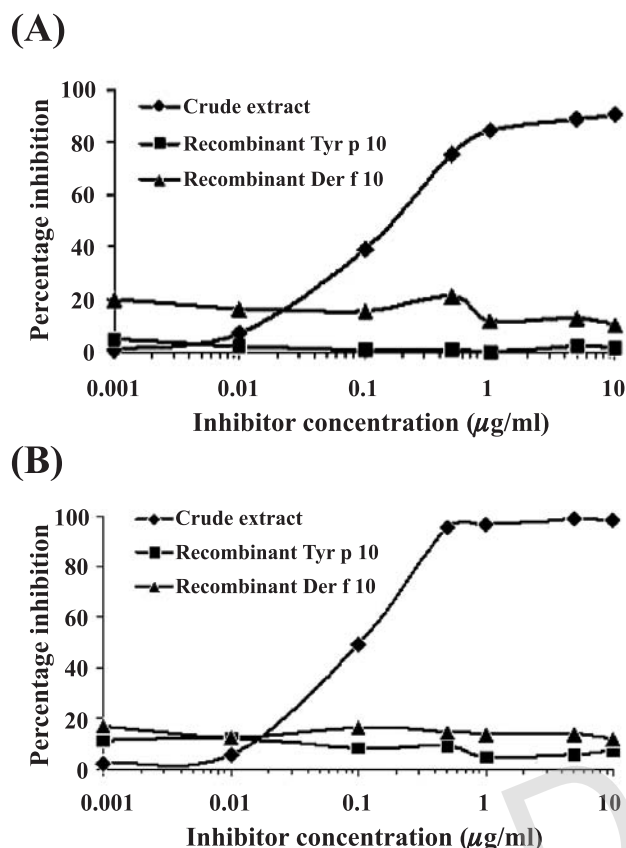


Figure 5. The ELISA inhibition of *T. putrescentiae* specific IgE with whole body extract and recombinant tropomyosins.

In summary, we have cloned the cDNA sequence encoding the Tyr p 10 allergen which shows 64-94% shared amino acid identity with other allergenic tropomyosins. The recombinant protein was detected in 12.5% of dust mite allergic sera tested and showed limited cross-reactivity. The cross-reactivity between house dust mite and storage mite seems to be influenced largely by other strong IgE binding molecules. The present study indicates that it may not be enough to use only one of these highly conserved allergens as diagnostic or therapeutic agents.

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