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## Allergenic Characterization of Tropomyosin from the Dusky Brown Cockroach, *Periplaneta fuliginosa*

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Household arthropods are one of the most common causes of allergic diseases. Four species of cockroaches are found to reside in Korean homes, but published work deals almost exclusively with the German and American cockroaches. This study was undertaken to investigate the cross-reactive allergenic components of the dusky brown cockroach, *Periplaneta fuliginosa*. Enzyme-linked immunosorbent assay (ELISA) inhibition and immunoblot analyses for the dusky brown cockroach were performed with *Blattella germanica* and *Dermatophagoides farinae* allergic sera. cDNA encoding tropomyosin, which is a well known cross-reactive pan-allergen, was cloned by reverse transcriptase PCR, and recombinant protein was produced by using a pET-28b expression system. Native tropomyosin was purified by ammonium sulfate fractionation and electroelution. The immunoglobulin E (IgE) reactivities of native and recombinant tropomyosins were compared by an ELISA inhibition study. All 30 sera tested showed *P. fuliginosa*-specific IgE, and the IgE-binding reactivity of the *P. fuliginosa* extract was inhibited as much as 79.4% by a *B. germanica* extract and as much as 63.3% by a *D. farinae* extract. The deduced amino acid sequence of cloned cDNA was identical with that of *Periplaneta americana* tropomyosin (98.5% nucleotide sequence identity). Seven of 26 (26.9%) allergic sera had IgE specific for recombinant protein, and the maximum inhibition of *P. fuliginosa*-specific IgE achieved with recombinant tropomyosin was 37.7% at an inhibitor concentration of 10  $\mu$ g/ml. Native tropomyosin inhibited the binding of IgE to the *P. fuliginosa*, *B. germanica*, and *D. farinae* extracts by 65.0, 51.8, and 39% at an inhibitor concentration of 1  $\mu$ g/ml. *P. fuliginosa* appears to possess allergens that are highly cross-reactive with allergens of *B. germanica* and *D. farinae*. Tropomyosin was found to be a major allergenic component accounting for the cross-reactivity between cockroaches and dust mites.

Invertebrates which infest homes and come into close contact with humans are major causative agents of allergy in susceptible individuals. The prevalence of cockroach-specific immunoglobulin E (IgE) antibodies has been found to be second only to that of antibodies specific to the house dust mite and to constitute a significant risk factor for acute asthma (27). Four cockroach species, the German (*Blattella germanica*) (63 of 174 [36.2%]), the American (*Periplaneta americana*) (58 of 174 [33.3%]), the Japanese (*Periplaneta japonica*) (2 of 174 [1.1%]), and the dusky brown (*Periplaneta fuliginosa*) (3 of 174 [1.7%]) cockroach, have been found to infest Korean homes (16). The dusky brown cockroach is a peridomestic pest distributed throughout much of the southeastern United States, Japan, and southeast Asia and has become increasingly important because this species infests shipping containers aboard airplanes, cargo ships, and semi-truck trailers (3). However, the literature on cockroach allergens is biased toward two species, the German cockroach and the American cockroach, from a total of more than 50 recorded species.

To date, six different kinds of allergens have been reported for the German cockroach: Bla g 1, a protein of unknown

function with tandem repeats (14, 28); Bla g 2, a protein whose primary sequence is homologous to that of aspartic proteinases but which is without enzymatic activity (4, 29); Bla g 4, a calcium binding protein (5); Bla g 5, a glutathione *S*-transferase (6); Bla g 6, troponin C (7); and Bla g 7, tropomyosin (17). Only three allergens from the American cockroach have been cloned: Per a 1, a protein of unknown function which is cross-reactive with Bla g 1 (24); Per a 3, an insect storage protein related to arylphorin (36); and Per a 7, tropomyosin (8). No detailed studies have been performed on other cockroach species, which may also represent sources of indoor allergens.

Tropomyosin is a family of closely related proteins present in muscle and nonmuscle cells. Interestingly, tropomyosin is also one of the best-known shellfish allergens (23). Because it shows high cross-reactivity among shellfish and arthropods, tropomyosin is thought to be an invertebrate pan-allergen (32).

In this study, we tried to characterize the tropomyosin from the dusky brown cockroach and produced a recombinant tropomyosin to determine the relevance of its cross-reactivity with other well-known allergens.

### MATERIALS AND METHODS

**Subjects and serum samples.** Human allergic serum samples were obtained from patients attending the allergy clinic at Severance Hospital, Yonsei University, Seoul, Korea. The diagnosis of allergy was based on a case history and skin prick testing. Sera from the patients were tested for the presence of IgE anti-

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bodies against *Dermatophagoides farinae* and *B. germanica* by using the Uni-CAP system (Pharmacia, Uppsala, Sweden) (11). Sera with CAP results higher than 0.7 kU/liter were used for the study of *P. fuliginosa* allergy ( $n = 30$ ; age range, 9 to 75 years; average age, 33 years). A serum pool from 13 healthy people was used as a negative control.

**Preparation of crude extracts.** Thirty grams of live or frozen cockroaches or mites was pulverized in liquid nitrogen. The sample was defatted in 200 ml of ethyl ether and ethyl acetate (1:1, by volume) and extracted with slow overhead stirring at 4°C overnight in phosphate-buffered saline (PBS), pH 7.4, containing 6 mM 2-mercaptoethanol and 1/1,000 volume of protease inhibitor set III (Calbiochem, San Diego, Calif.). Then 1 mg of 1-phenyl-3-(2-thiazolyl)-2-thiourea (Sigma, St. Louis, Mo.)/ml was added to prevent melanization. The extract was then centrifuged at  $10,000 \times g$  for 30 min at 4°C, and the supernatant was finally filtered through a 0.22- $\mu$ m-pore-size filter (Millipore, Bedford, Mass.)

**Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting.** Proteins separated on a 10% gel were electroblotted in the XCELL II Blot module (NOVEX, San Diego, Calif.) according to the manufacturer's instructions. After blotting, the nitrocellulose membrane (OSMONICS Inc., Westborough, Mass.) was blocked with 3% skim milk in PBS containing 0.05% Tween 20, cut into 2- to 3-mm-long strips, and incubated overnight with serum samples diluted 1/3 in PBS. The strips were subsequently incubated with alkaline phosphate-conjugated monoclonal anti-human IgE (1:1,000 dilution in PBS containing 0.05% Tween 20 and 1% bovine serum albumin) and visualized by using nitroblue tetrazolium (NBT)-3-bromo-4-chloro-5-indolylphosphate (BCIP) as a substrate (Promega, Madison, Wis.).

**Molecular cloning of the cDNA encoding tropomyosin.** Total RNA was isolated from 100 mg of dusky brown cockroach by using TRIzol reagent (Gibco BRL, Rockville, Md.). First-strand cDNA was synthesized from 5  $\mu$ g of total RNA by using an oligo(dT) ( $T_{18}$ ) primer. Oligodeoxynucleotide primers, which were designed for cDNA amplification of German cockroach tropomyosin (17), were used to clone the tropomyosin of *P. fuliginosa*. The primer sequences used to produce the full-length cDNA were as follows: forward primer; 5'-ATGGA TGCCATCAAGAAGAAG-3'; reverse primer; 5'-GTTTAGTTGCCAATAAG TTCGG-3'. Five microliters of single-stranded cDNA was mixed with the primers in a standard PCR mixture and amplified with *Taq* DNA polymerase under the following conditions. After initial denaturation at 95°C for 5 min, the samples were subjected to 35 cycles at 94°C for 30 s, 53°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 9 min. The PCR-amplified fragment was isolated from a 1% agarose gel and ligated to the pGEM-T Easy vector (Promega).

**Nucleotide sequence determination.** Nucleotide sequences were determined with the ABI PRISM Dye Terminator Cycle Sequencing Ready reaction kit (Perkin-Elmer Applied Biosystems, Foster City, Calif.) and run on an ABI 373A DNA sequencer (Perkin-Elmer Applied Biosystems) by using commercial and sequence based primers. Sequences were analyzed by using the BLAST program.

**Expression and purification of recombinant tropomyosin.** The open reading frame of tropomyosin was amplified by reverse transcriptase PCR, and the cDNA sequence cloned into the pGEM-T Easy vector was subcloned into the EcoRI-cut site of the pET-28b vector (Novagen, Madison, Wis.). The resultant sequence had an additional 35 amino acids at its N terminus.

The recombinant tropomyosin was expressed in *Escherichia coli* BL21(DE3). Isopropyl-1-thiogalactopyranoside (IPTG; 1 mM) was added to the bacterial culture, which was then grown at 37°C in Luria-Bertani broth to an optical density at 600 nm ( $OD_{600}$ ) of 0.5 in order to induce the expression of recombinant protein. The culture was harvested after being induced for 4 h. The pellet was resuspended in lysis buffer (10 mM imidazole, 300 mM NaCl, 50 mM  $\text{NaH}_2\text{PO}_4$  [pH 8.0]) and lysed by using a French press. Recombinant tropomyosin was purified by using nitrilotriacetic acid-agarose (QIAGEN, Valencia, Calif.) according to the manufacturer's protocol under native conditions.

**Purification of native tropomyosin.** Live cockroaches (60 g) were homogenized in 200 ml of 6 mM 2-mercaptoethanol in a blender at 4°C. All subsequent steps were performed at 4°C unless otherwise noted. The homogenate was expressed through three layers of cheesecloth. The filtered homogenate was mixed with 20 volumes (4 liters) of ethanol. The supernatant was allowed to stand for 1 h and then centrifuged (centrifuge from Sorvall, Albertville, Minn.) for 30 min at  $2,000 \times g$  to collect the sediment. The supernatant was discarded, and the sediment was resuspended in ether and then air dried. The dried residue was extracted with gentle overhead stirring overnight in a solution containing 1 M KCl, 10 mM Tris, 6 mM 2-mercaptoethanol, 1 mg of 1-phenyl-3-(2-thiazolyl)-2-thiourea (Sigma)/ml, 0.5 mM EDTA, and protease inhibitor set III (Calbiochem) (pH 7.5). The insoluble residue was removed by centrifugation at  $20,000 \times g$  for 30 min. The clear supernatant was fractionated with 40 to 55% ammonium sulfate saturation. The precipitate recovered by centrifugation at  $30,000 \times$

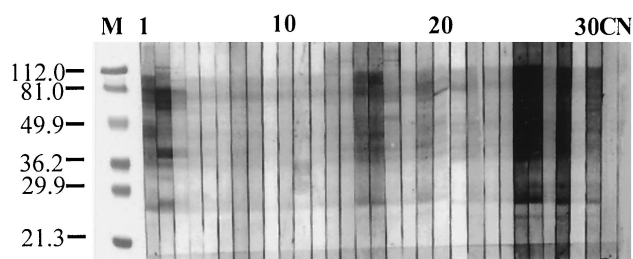


FIG. 1. *P. fuliginosa* extracts were blotted onto nitrocellulose membranes and probed with sera from 30 allergic patients, followed by anti-human IgE. Positions of molecular mass markers are given on the left. C, buffer control without serum addition; N, normal pooled sera.

$g$  for 30 min was subjected to separation on a 4-to-20% polyacrylamide gradient gel. Further purification was achieved by electroelution using a model 422 Electro-Eluter (Bio-Rad, Hercules, Calif.).

**Specific IgE binding to tropomyosin.** The prevalence of specific IgE antibodies against tropomyosin was determined by an enzyme-linked immunosorbent assay (ELISA). Briefly, each well of the microtiter plates was coated with 200  $\mu$ l of recombinant tropomyosin. Alkaline phosphatase-conjugated mouse monoclonal anti-human IgE was then diluted 1:1,000 in PBS-0.05% Tween 20 containing 1% bovine serum albumin (Sigma). For color development, 100  $\mu$ l of *para*-nitrophenyl phosphate (1 mg/ml) (Sigma) was added and absorbance was measured by using an automatic microplate reader (TECAN, Salzburg, Austria). Assays were carried out in triplicate, and the mean absorbance level of the serum samples from 13 healthy controls plus 2 standard deviations was used as the cutoff value.

**ELISA inhibition test.** Polystyrene microtiter plates were coated with 100  $\mu$ l of a 20- $\mu$ g/ml *P. fuliginosa*, *B. germanica*, or *D. farinae* extract in 50 mM carbonate buffer (pH 9.6) overnight at 4°C. The 1:4-diluted serum was preincubated with various quantities of the crude extract, purified protein, or recombinant protein for 2 h at room temperature and overnight at 4°C. After a wash, each well of the microtiter plate was blocked with 200  $\mu$ l of 3% skim milk in PBS containing 0.05% Tween 20 for 1 h at room temperature. Fifty microliters of the preincubated serum was then incubated for 1 h at room temperature. Specific IgE was detected by incubation with 50  $\mu$ l of 1:1,000-diluted biotinylated goat anti-human IgE (Vector, Burlingame, Calif.), followed by streptavidin-peroxidase (Sigma) diluted 1:1,000, for 1 h and 30 min, respectively. The signal was developed by adding 3,3',5,5'-tetramethyl-benzidine (TMB; Kirkegaard & Perry Laboratories, Gaithersburg, Md.) (32) at a concentration of 0.4 g/liter.

**Nucleotide sequence accession number.** The nucleotide sequence of dusky brown cockroach tropomyosin has been deposited at GenBank under accession number AF454866.

## RESULTS

**IgE recognition profile of *P. fuliginosa* allergens.** Thirty allergic sera sensitized with both *D. farinae* and *B. germanica* were used to examine the prevalence of IgE recognition and the allergen profile of *P. fuliginosa*. All 30 allergic sera had IgE antibodies against *P. fuliginosa* (Fig. 1). The molecular weight profiles of the IgE-binding components were significantly different for different patients' sera, and a number of various proteins were recognized. All patients' sera revealed weak or strong IgE reactivity to high-molecular-weight components above 70 kDa. More than one-third of the sera exhibited IgE reactivity to 25-kDa components (serum samples 1, 2, 3, 15, 16, 19, 25, 26, 28, and 30) and to components between 36 and 49 kDa (serum samples 1, 2, 3, 11, 15, 16, 17, 19, 22, 25, 26, 28, and 30).

**Cross-reactivity with *B. germanica* and *D. farinae*.** An inhibition ELISA was performed to investigate the cross-reactivity of *P. fuliginosa* with *B. germanica* and *D. farinae* (Fig. 2). *P. fuliginosa*-specific IgE binding reactivity was inhibited as much

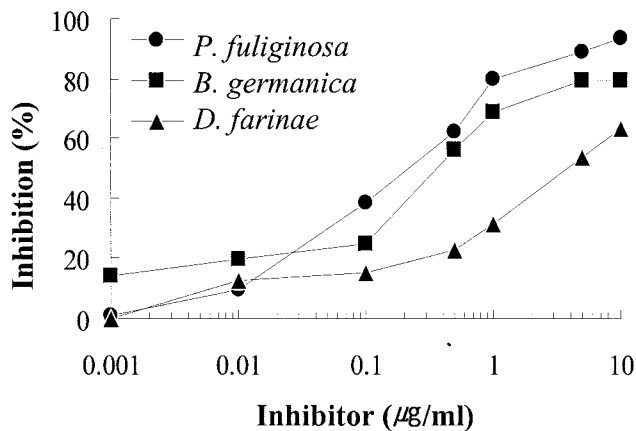


FIG. 2. Inhibition ELISA of *P. fuliginosa* with *B. germanica* and *D. farinae*.

as 79.4% by *B. germanica* extract and as much as 63.3% by *D. farinae* extract.

**cDNA cloning of tropomyosin.** A single fragment of approximately 850 bp was produced by reverse transcriptase PCR, cloned into the pGEM-T Easy vector, and sequenced. The cloned sequence encoded 284 amino acids with an estimated molecular mass of 32,739 Da and an isoelectric point of 4.59 (Fig. 3). Dusky brown cockroach tropomyosin was found to have the same amino acid sequence as American cockroach tropomyosin and to have 98.5% nucleotide sequence identity with both American and German cockroach tropomyosins. Differences between the cDNAs of *P. fuliginosa* tropomyosin

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ATGGATGCCATCAAGAAGAAGATGCAGCGGATGAAGCTGGAGAAGGACAACGCGATGGAC 60
M D A I K K K M Q A M K L E K D N A M D
CGTGCCCTTCTCGCAGCAGCAGGCCGCGCAGCAACCTTAGGGCCGAGAAGGCTGAG 120
R A L L C E Q Q A R D A N L R A E K A E
GAGGAGGCGCGCTCCCTGCAGAAGAAGATCCAGCAGATGAGAATGATCTGGATCAGACC 180
E E A R S L Q K K I Q Q I E N D L D Q T
ATGGAGCAGCTGATGCAGGTAACGCCAAGCTGGAGGAGAAGGACAAGGCCCTCCAGAAC 240
M E Q L M Q V N A K L D E K D K A L Q N
GCTGAGAGTGAAGTTGCTGCCCTGAACCCGCTATCCAGCTGCTGGAGGAGGACCTCGAG 300
A E S E V A A L N R R I Q L L E E D L E
AGGTTGAGGAAAGATTGGCCACTGCCACTGCCAACTGGCCGAGGCTTCACAGGCTGCC 360
R S E E R L A T A T A K L A E A S Q A A
GATGAGTCCGAGCGAGCCGTAAGATTCTGAATCCAAGGCCCTGGCAGATGAAGAGCCG 420
D E S E R A R K I L E S K G L A D E E R
ATGGAGCAGCTGGAGAACCAGCTGAAGAGGCCAGGTTTCATGGCTGAAGAAGCAGACAAG 480
M D A L E N Q L K E A R F M A E E A D K
AAATATGATGAGTTGCACGTAAGTTGCCATGGTTGAGGCCGACTTGGAGAGCAGAG 540
K Y D E V A R K L A M V E A D L E R A E
GAACGTTGCGAGAGTGGTGAATCCAAAATTGTAGAGCTTGAAGGAGGAGCTCCGCTGTGTC 600
E R A E S G E S K I V E L E E E L R V V
GGCAACACCTGAAGTCCCTTGAGGTTGCTGAAGAGAAGGCCAACCTGCGTGAAGAAGCG 660
G N N L K S L E V S E E K A N L R E E A
TACAACGACGAGATTAAAGACCTTGAACCTGACAGGCTAAAGGAGGCTGAGGCCGCTGAG 720
Y K Q Q I K T L T T R L K E A E A R A E
TTCGCTGAGAGGTCCTGCAGAGCTGAGAAGGAGGTTGACAGGCTTGAAGATGAATG 780
F A E R S V Q K L Q K E V D R L E D E L
GTACACGAGAAGGAGAAGTACAAGTTCAATTTGTGACGATCTGATATGACTTTCACCGAA 840
V H E K E K Y K F I C D D L D M T F T E
CTTATGGCAACTAAAC 957
L I G N
    
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FIG. 3. Nucleotide and deduced amino acid sequences of dusky brown cockroach tropomyosin.

and *P. americana* tropomyosin were found at 13 positions: positions 6 (T to C), 9 (C to G), 63 (T to C), 175 (T to C), 285 (G to A), 313 (A to C), 345 (G to A), 366 (G to A), 417 (G to A), 480 (G to A), 633 (A to G), 658 (C to A), and 816 (C to T). The GenBank data search program revealed that *P. fuliginosa* tropomyosin shared the highest identity with the other cockroach tropomyosins (100% with *P. americana* and 98.2% with *B. germanica*). It was also found to have high homology with other allergenic tropomyosins such as those of shrimp (84 to 81%) and house dust mites (80%) (Fig. 4).

*P. fuliginosa* tropomyosin was designated Per f 7 in accordance with the World Health Organization/International Union of Immunological Societies (WHO/IUIS) system of allergen nomenclature (20).

**Expression and purification of recombinant tropomyosin.** Per f 7 cDNA was subcloned into the pET-28b expression vector, and the molecular weight of the protein expressed was determined by SDS-PAGE (Fig. 5a). Its estimated molecular mass was 36.407 kDa, because it has a histidine-tag at the N terminus, but the recombinant protein produced a band of 42 kDa. The yield of the purified protein was 6.475 mg/liter as measured by a Bradford assay (Bio-Rad).

**Purification of native tropomyosin.** Native tropomyosin was purified by ammonium sulfate fractionation and electroelution. The yield of native tropomyosin (649 µg/ml) from live cockroaches was 1.1%. The purified tropomyosin migrated as a 36-kDa band on SDS-PAGE (Fig. 5b).

**IgE reactivity of recombinant tropomyosin.** IgE-binding frequency against recombinant Per f 7 were determined by ELISA. The data showed that 7 of the 26 serum samples tested (26.9%) were positive (Table 1). This result implies that other proteins of *P. fuliginosa* could be more allergenic.

**Inhibition of specific IgE binding to crude extracts.** The crude extract of *P. fuliginosa* showed a maximum inhibition of 83.1%, and recombinant tropomyosin showed 37.7% inhibition, at inhibitor concentrations of 10 µg/ml (Fig. 6). Inhibition studies were also performed with crude extracts of *B. germanica* and *D. farinae* in order to investigate the cross-reactivity of tropomyosin. All three extracts (*P. fuliginosa*, *B. germanica*, and *D. farinae*) inhibited the IgE response to various degrees. Both the *P. fuliginosa* extract and native tropomyosin inhibited the *P. fuliginosa* extract 65% at an inhibitor concentration of 1 µg/ml. Recombinant tropomyosin, however, inhibited only 25.7% of the IgE response to the *P. fuliginosa* extract (Fig. 6a). At the same time, the *P. fuliginosa* extract and native tropomyosin inhibited the binding of IgE to the *B. germanica* extract to about the same degree, 51.1 and 51.8%, respectively, while recombinant tropomyosin inhibited this binding 23.2% at an inhibitor concentration of 1 µg/ml (Fig. 6b). Analogous experiments were performed with the *D. farinae* extract. The inhibition of IgE binding to *D. farinae* reached 33.7, 39.0, and 25.8% with the *P. fuliginosa* extract, native tropomyosin, and recombinant tropomyosin, respectively, at an inhibitor concentration of 1 µg/ml (Fig. 6c).

**DISCUSSION**

IgE-mediated allergy is the most common cause of hypersensitivity (19). Cross-reactivity by IgE is of interest because it may reflect the pattern of clinical sensitivities (1). In this study,

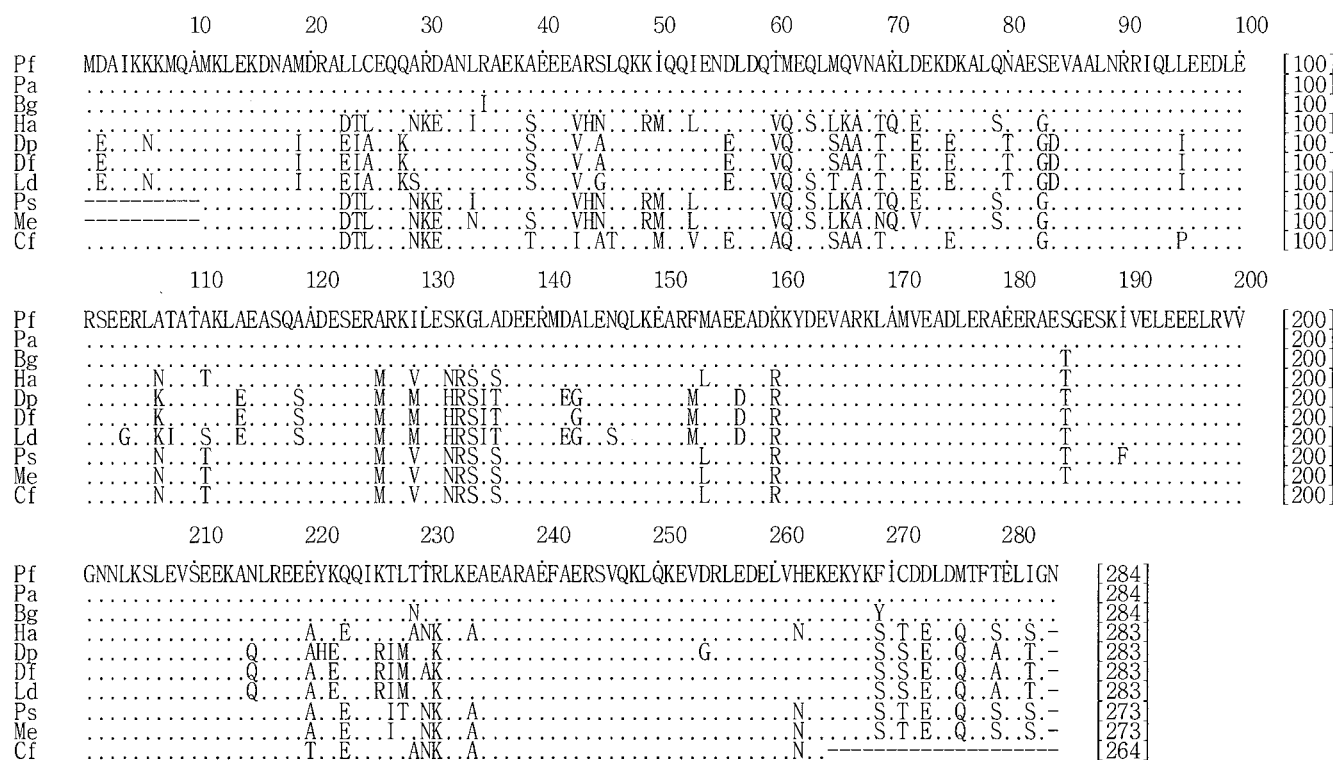


FIG. 4. Comparison of *P. fuliginosa* tropomyosin with different tropomyosins. Pf, *P. fuliginosa*; Pa, *P. americana* (accession number AF106961); Bg, *B. germanica* (AF260897); Ps, *Panulirus stimpsoni* (AF030063); Me, *Metapenaeus ensis* (U08008); Ha, *Homarus americanus* (AF034954); Cf, *Charybdis feriatius* (AF061783); Dp, *D. pteronyssinus* (AF016278); Df, *D. farinae* (D17682); Ld, *Lepidoglyphus destructor* (AJ250096).

we evaluated the allergenic cross-reactivities of *P. fuliginosa* with *D. farinae* and *B. germanica* by inhibition ELISA. The results suggest that these three species share allergenic epitopes. Several lines of evidence suggest that a 36- to 40-kDa antigen, possibly tropomyosin, could be the main cross-sensitization link between disparate species (13, 26, 34). In particular, it has been reported that IgE antibodies to tropomyosin could be induced during mite immunotherapy (35).

Only 37.7% of the *P. fuliginosa* crude extract was inhibited in the present study by recombinant tropomyosin, even though it had the same amino acid sequence as Per a 7, which is a

TABLE 1. IgE reactivity of human sera against recombinant tropomyosin

Serum sample	Sex <sup>a</sup>	Age (yr)	Diagnosis	Total IgE (U/ml)	OD <sub>405</sub> <sup>b</sup>
P01	M	27	Allergic asthma	1,035	0.203
P02	F	43	Allergic asthma	151	0.167
P03	F	26	Allergic rhinitis	236	0.185
P04	F	40	Allergic asthma	586	0.163
P05	M	47	Allergic urticaria	117	0.382
P06	M	28	Allergic urticaria	122	0.142
P07	F	33	Allergic asthma	426	0.153
P08	F	40	Allergic asthma	1,076	0.137
P09	M	9	Allergic asthma	1,790	0.164
P10	M	15	Atopic dermatitis	506	0.471
P11	M	36	Cough	425	0.137
P12	M	17	Allergic rhinitis	1,418	0.204
P13	M	34	Allergic urticaria	465	0.183
P14	M	75	COPD <sup>c</sup>	568	0.130
P15	M	28	Allergic asthma	1,372	0.170
P16	M	62	Allergic asthma	5,000	0.144
P17	F	20	Atopic dermatitis	5,000	0.224
P18	M	34	Allergic urticaria	1,775	0.189
P19	F	57	Allergic asthma	1,106	0.153
P20	M	21	Atopic dermatitis	3,133	0.179
P21	M	20	Allergic asthma	1,205	0.188
P22	F	31	Atopic dermatitis	1,079	0.232
P23	M	33	Atopic dermatitis	1,089	0.171
P24	F	28	Angioedema	1,774	0.412
P25	F	50	Allergic rhinitis	149	0.296
P26	M	31	Eosi nophilia	5,000	0.257

<sup>a</sup> M, male; F, female.

<sup>b</sup> The cutoff value was 0.213.

<sup>c</sup> COPD, chronic obstructive pulmonary disease.

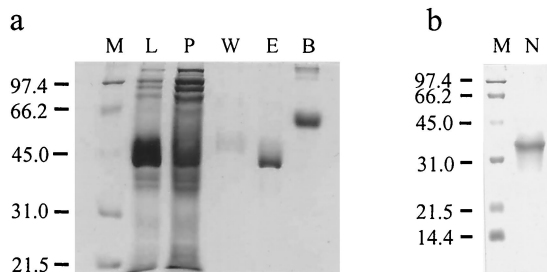


FIG. 5. Purification of native and recombinant tropomyosins. Proteins were separated under reducing conditions on 10% acrylamide gels and stained with Coomassie brilliant blue. Lanes: M, molecular size markers (in kilodaltons); L, total cell lysate of *E. coli* BL21 cells bearing recombinant Per f 7 after IPTG induction; P, fraction passed through the column; W, fraction washed from the column; E, fraction eluted; B, bovine serum albumin; N, native tropomyosin.

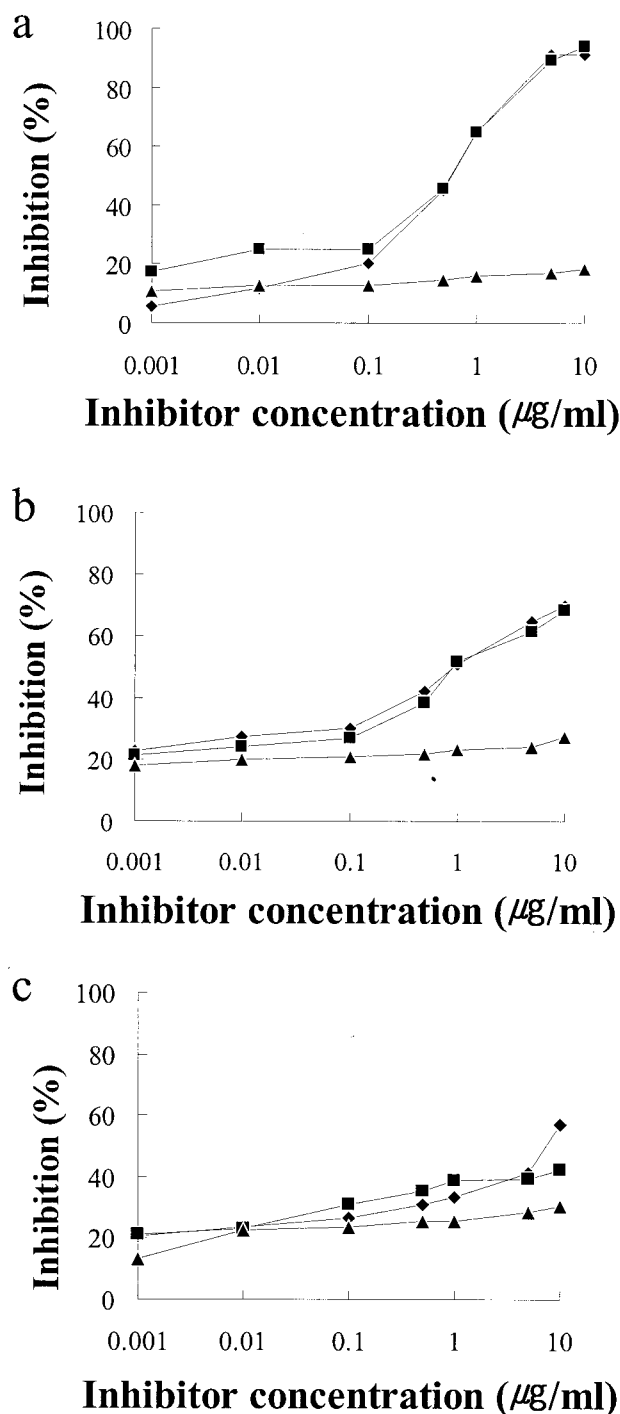


FIG. 6. ELISA inhibition analysis. IgE antibody reactivities of serum pools to crude extracts of *P. fuliginosa* (a), *B. germanica* (b), and *D. farinae* (c) were inhibited with a *P. fuliginosa* crude extract (◆), native tropomyosin (■), and recombinant tropomyosin (▲).

well-known highly cross-reactive allergen. IgE-binding frequencies of recombinant tropomyosin are not consistent: 81% in *Chironomus kiiensis* (18), 80.6% in *D. farinae* (2), 41.4% in *P. americana* (8), 29% in *Blomia tropicalis* (37), and 5.6% in *Dermatophagoides pteronyssinus* (9).

In the study using native tropomyosin, it was found that tropomyosin is a major component accounting for cross-reactivity. Even sequences that differ in one or more amino acids were reported to still recognize and bind IgE antibodies from shrimp allergic sera, although with somewhat different intensity, in a study of tropomyosin epitopes (10). The large differences between native and recombinant tropomyosin are in agreement with a previous report on *D. farinae* tropomyosin (2).

Tropomyosin is a family of closely related proteins with multiple functions: muscle contraction (21, 22), transport of mRNA (12), and mechanical support of cells (15). At least 18 isoforms are known to have been generated by alternative RNA splicing in mammalian cells (33). Moreover, a small change in the amino acid sequence of isoforms could influence the IgE-binding reactivity of the allergen (25). In addition, differences between native and recombinant tropomyosin may contribute to the different allergenicities, and fusion proteins or the inappropriate folding of recombinant proteins may influence IgE-binding reactivity. Also, patient group differences, such as different genetic backgrounds, may influence IgE-binding frequency.

More effort is needed to elucidate the causes of different allergenicities, but the relevance of tropomyosin should not be underestimated, because of its strong cross-reactivity. This study indicates that allergenic cross-reactivity also involves allergens other than tropomyosin. It is necessary that these other allergens be examined in order to fully understand the cross-reactivity of cockroach allergens. Studies on the relationship between structure and allergenicity (30) in conjunction with patient genetics could provide a basis for understanding allergic disease and may allow the creation of new therapeutic strategies (31).

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#### REFERENCES

1. Aalberse, R. C., J. H. Akkerdaas, and R. van Ree. 2001. Cross-reactivity of IgE antibodies to allergens. *Allergy* 56:478–490.
2. Aki, T., T. Kodama, A. Fugikawa, K. Miura, S. Shigeta, T. Wada, T. Jyo, Y. Murooka, S. Oka, and K. Ono. 1995. Immunochemical characterization of recombinant and native tropomyosin as a new allergen from the house dust mite, *Dermatophagoides farinae*. *J. Allergy Clin. Immunol.* 96:74–83.
3. Appel, A. G., and L. M. Smith II. 2002. Biology and management of the smokybrown cockroach. *Annu. Rev. Entomol.* 47:33–55.
4. Arruda, L. K., L. D. Vailes, B. J. Mann, J. Shannon, J. W. Fox, and T. S. Vedvick. 1995. Molecular cloning of a major cockroach (*Blattella germanica*) allergen, Bla g 2. Sequence homology to the aspartic proteases. *J. Biol. Chem.* 270:19563–19568.
5. Arruda, L. K., L. D. Vailes, M. L. Hayden, D. C. Benjamin, and M. D. Chapman. 1995. Cloning of cockroach allergen, Bla g 4, identifies ligand binding proteins (or calycons) as a cause of IgE antibody responses. *J. Biol. Chem.* 270:31196–31201.
6. Arruda, L. K., L. D. Vailes, T. A. E. Platts-Mills, M. L. Hayden, and M. D. Chapman. 1997. Induction of IgE antibody responses by glutathione S-transferase from the German cockroach (*Blattella germanica*). *J. Biol. Chem.* 272:20907–20912.
7. Arruda, L. K., L. D. Vailes, D. C. Benjamin, and M. D. Chapman. 1995. Molecular cloning of German cockroach (*Blattella germanica*) allergens. *Int. Arch. Allergy Immunol.* 107:295–297.
8. Asturias, J. A., N. Gomez-Bayon, M. C. Arilla, A. Martinez, R. Palacios, F. Sanchez-Gascón, and J. Martinez. 1999. Molecular characterization of American cockroach tropomyosin (*Periplaneta americana* allergen 7), a cross-reactive allergen. *J. Immunol.* 162:4342–4348.
9. Asturias, J. A., M. C. Arilla, N. Gomez-Bayon, A. Martinez, J. Martinez, and R. Palacios. 1998. Sequencing and high level of expression in *Escherichia coli*

- of the tropomyosin allergen (Der p 10) from *Dermatophagoides pteronyssinus*. *Biochim. Biophys. Acta* **1397**:27–30.
10. Ayuso, R., G. Reese, S. Leong-Kee, M. Plante, and S. B. Lehrer. 2002. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. *Int. Arch. Allergy Immunol.* **129**:38–48.
  11. Bousquet, J., P. Chanez, I. Chanal, and F. B. Michel. 1990. Comparison between RAST and Pharmacia CAP system: a new automated specific IgE assay. *J. Allergy Clin. Immunol.* **85**:1039–1043.
  12. Erdelyi, M., A. M. Michon, A. Guichet, J. B. Glotzer, and A. Ephrussi. 1995. Requirement for *Drosophila* cytoplasmic tropomyosin in *oskar* mRNA localization. *Nature* **377**:524–527.
  13. Fernandez-Caldas, E., S. Quirce, F. Maranon, M. L. Diez Gomez, H. G. Botella, and R. L. Roman. 1998. Allergenic cross-reactivity between third stage larvae of *Hysterothylacium aduncum* and *Anisakis simplex*. *J. Allergy Clin. Immunol.* **101**:554–555.
  14. Helm, R., G. Cockrell, J. S. Stanley, R. J. Brenner, W. Burks, and G. A. Bannon. 1996. Isolation and characterization of a clone encoding a major allergen (Bla g Bd90K) involved in IgE-mediated cockroach sensitivity. *J. Allergy Clin. Immunol.* **98**:172–180.
  15. Hitt, A. L., and E. J. Luna. 1994. Membrane interactions with the actin cytoskeleton. *Curr. Opin. Cell Biol.* **6**:120–130.
  16. Jeong, K. Y., I. Y. Lee, J. Lee, H. I. Ree, C. S. Hong, and T. S. Yong. Effectiveness of health education for the control of house dust mites and cockroaches in Seoul, Korea. *Med. Vet. Entomol.*, in press.
  17. Jeong, K. Y., J. Lee, I. Y. Lee, H. I. Ree, C. S. Hong, and T. S. Yong. 2003. Allergenicity of recombinant Bla g 7, German cockroach tropomyosin. *Allergy* **58**:1059–1063.
  18. Jeong, K. Y., H. Y. Yum, I. Y. Lee, H. I. Ree, C. S. Hong, D. S. Kim, and T. S. Yong. 2004. Molecular cloning and characterization of tropomyosin, a major allergen of *Chironomus kiiensis*, a dominant species of non-biting midges in Korea. *Clin. Diagn. Lab. Immunol.* **11**:320–324.
  19. Johansson, S. G. O., J. O. Hourihane, J. Bousquet, C. Brujnzeel-Koomen, S. Dreborg, T. Haahtela, M. L. Kowalski, N. Mygind, J. Ring, P. van Cauwenberge, M. van Hage-Hamsten, and B. Wuthrich. 2001. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* **56**:813–824.
  20. King, T. P., D. Hoffman, H. Lowenstein, D. G. Marsh, T. A. E. Platts-Mills, and W. Thomas. 1994. Allergen nomenclature. *Int. Arch. Allergy Immunol.* **105**:224–233.
  21. Lehman, W., R. Craig, and P. Vibert. 1994. Ca<sup>2+</sup>-induced tropomyosin movement in *Limulus* thin filaments revealed by three-dimensional reconstruction. *Nature* **368**:65–67.
  22. Lehman, W., P. Vibert, P. Uman, and R. Craig. 1995. Steric-blocking by tropomyosin visualized in relaxed vertebrate muscle thin filaments. *J. Mol. Biol.* **251**:191–196.
  23. Lehrer, S. B., R. Ayuso, and G. Reese. 2002. Current understanding of food allergens. *Ann. N. Y. Acad. Sci.* **964**:69–85.
  24. Melen, E., A. Pomes, L. D. Vailes, L. K. Arruda, and M. D. Chapman. 1999. Molecular cloning of Per a 1 and definition of the cross-reactive group 1 cockroach allergens. *J. Allergy Clin. Immunol.* **103**:859–864.
  25. Park, J. W., K. S. Kim, H. S. Jin, C. W. Kim, D. B. Kang, S. Y. Choi, T. S. Yong, S. H. Oh, and C. S. Hong. 2002. Der p 2 isoallergens have different allergenicity, and quantification with 2-site ELISA using monoclonal antibodies is influenced by the isoallergens. *Clin. Exp. Allergy* **32**:1042–1047.
  26. Pascual, C. Y., J. F. Crespo, S. San Martin, N. Ornia, N. Ortega, T. Caballero, M. Munoz-Pereira, and M. Martin-Esteban. 1997. Cross-reactivity between IgE-binding proteins from *Anisakis*, German cockroach, and chironomids. *Allergy* **52**:514–520.
  27. Pollart, S. M., M. D. Chapman, G. P. Fiocco, G. Rose, and T. A. E. Platts-Mills. 1989. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. *J. Allergy Clin. Immunol.* **83**:875–882.
  28. Pomes, A., L. D. Vailes, R. M. Helm, and M. D. Chapman. 2002. IgE reactivity of tandem repeats derived from cockroach allergen, Bla g 1. *Eur. J. Biochem.* **269**:3086–3092.
  29. Pomes, A., M. D. Chapman, L. D. Vailes, T. L. Blundell, and V. Dhanaraj. 2002. Cockroach allergen Bla g 2: structure, function, and implications for allergic sensitization. *Am. J. Respir. Crit. Care Med.* **165**:391–397.
  30. Pomes, A. 2002. Intrinsic properties of allergens and environmental exposure as determinants of allergenicity. *Allergy* **57**:673–679.
  31. Pomes, A., and M. D. Chapman. 2001. Can knowledge of the molecular structure of allergens improve immunotherapy? *Curr. Opin. Allergy Clin. Immunol.* **1**:549–554.
  32. Reese, G., R. Ayuso, and S. B. Lehrer. 1999. Tropomyosin: an invertebrate pan-allergen. *Int. Arch. Allergy Immunol.* **119**:247–258.
  33. Rethinasamy, P., M. Muthuchamy, T. Hewett, G. Boivin, B. Wolska, C. Evans, R. Solaro, and D. Wiczorek. 1997. Molecular and physiological effects of alpha-tropomyosin ablation in mouse. *Circ. Res.* **82**:116–123.
  34. Thomas, W. R., and W. Smith. 1998. House-dust-mite allergens. *Allergy* **53**:821–832.
  35. van Ree, R., L. Antonicelli, J. H. Akkerdaas, M. S. Garrtani, R. C. Aalberse, and F. Bonifazi. 1996. Possible induction of food allergy during mite immunotherapy. *Allergy* **51**:108–113.
  36. Wu, C. H., M. F. Lee, S. C. Liao, and S. F. Luo. 1996. Sequence analysis of cDNA clones encoding the American cockroach Cr-P1 allergens. *J. Biol. Chem.* **271**:17937–17943.
  37. Yi, F. C., N. Cheong, P. C. L. Shek, D. Y. Wang, L. Y. Chua, and B. W. Lee. 2002. Identification of shared and unique immunoglobulin E epitopes of the highly conserved tropomyosins in *Blomia tropicalis* and *Dermatophagoides pteronyssinus*. *Clin. Exp. Allergy* **32**:1203–1210.

## AUTHOR'S CORRECTION

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