

Identification and molecular characterization of *Charybdis feriatus* tropomyosin, the major crab allergen

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Background: Crab sensitivity is one of the most common seafood allergies. However, to date, there has been no report on the molecular characterization of crab allergens and no comparative analysis with other seafood allergens.

Objective: This study was undertaken to clone, identify, and determine the primary structure of a major IgE-reactive molecule in crab.

Methods: We constructed an expression cDNA library from a common crab, *Charybdis feriatus*. This library was then screened with the use of sera from subjects with a well-documented history of type I hypersensitivity reactions upon ingestion of crab. An IgE-reactive clone was chosen and subcloned into plasmids for nucleotide sequence determination and expression in *Escherichia coli*.

Results: We identified a 1-kb cDNA designated as Cha f 1.

Expression of Cha f 1 produces a 34-kd recombinant protein reactive to the IgE antibodies from patients with crab allergies but not from control subjects. Cha f 1 has an opening reading frame of 264 amino acids and demonstrates marked homology to the shrimp tropomyosin Met e 1. Absorption of allergic sera with Cha f 1 removes IgE reactivity to crab extract. Moreover, absorption of allergic sera with recombinant shrimp Met e 1 tropomyosin removes IgE reactivity to Cha f 1.

Conclusions: This 34-kd protein, designated as Cha f 1, is the first identified major allergen of crab. Nucleotide and amino acid comparison shows that this protein is the crab tropomyosin. The molecular basis of shrimp and crab allergy is readily demonstrated at the nucleotide and amino acid level. (J Allergy Clin Immunol 1998;102:847-52.)

Key words: Crab allergen, cDNA, Cha f 1, tropomyosin

IgE-mediated hypersensitive reactions to ingestion of shellfish are among the most serious forms of food allergies.¹ In the United States alone, more than 250,000 individuals are at risk of having hypersensitive responses upon

ingestion of shellfish²; the majority of them are allergic to multiple shellfish, such as shrimp, crab, and lobster. Sensitized individuals can develop urticaria, angioedema, laryngospasm, asthma, and life-threatening anaphylaxis.³⁻⁶ For many years, reports on the identification of crustacean allergens were limited to the penaeid shrimp. Hoffmann et al⁷ demonstrated the presence of heat-stable and heat-labile allergens in shrimp. The heat-stable allergen, namely antigen II, was identified as a 38-kd protein. Nagpal et al⁸ reported the isolation of 2 heat-stable allergens from *Penaeus indicus*, designated as Sa-I and Sa-II; Sa-II, with a molecular weight of 34 kd, was identified to be a peptide of 301 amino acid residues. A comparison of amino acid compositions suggests that antigen-II and Sa-II are the same allergenic material, representing the major shrimp allergen.^{3,4} It was not until recently that the shrimp allergen was identified as the muscle protein tropomyosin.⁹⁻¹¹

The cDNA and amino acid sequences of the major shrimp allergen Met e 1 derived from *Metapenaeus ensis* (greasyback shrimp) have been reported. The nucleotide sequence of Met e 1 cDNA clone reveals an open reading frame of 281 amino acid residues, coding for a protein of 34 kd; comparison of the Met e 1 amino acid sequence with the GenBank database shows that Met e 1 is of significant homology to *Drosophila* and mammalian tropomyosin.^{12,13} Shanti et al⁹ also demonstrated an 86% homology between Sa-II and tropomyosin by comparing amino acid sequences of different peptides generated by proteolysis of Sa-II. In addition, Reese et al¹⁴ reported the primary structure of the *Penaeus aztecus* (brown shrimp) tropomyosin, Pen a 1, and demonstrated its allergenicity with a serum pool of subjects allergic to shrimp.¹⁴ Thus it is now well established that tropomyosin is the major shrimp allergen. However, biochemical data and molecular identity of allergens from other crustaceans are lacking.

Recently, by using recombinant shrimp tropomyosin Met e 1, our laboratory demonstrated that Met e 1 can absorb all the IgE reactivity of crustacean allergy sera against a 38-kd protein in both lobster and crab by immunoblotting, providing evidence that tropomyosin is a common allergen among crustaceans.¹⁵

Hefle et al¹⁶ reported the presence of multiple IgE-reactive proteins of 25 to 45 kd and 5 IgE proteins of 14 kd or less in snow crab meat and cooking water extracts. Moreover, they reported that 5 of 18 serum samples had IgE binding to single bands at 38 to 41 kd, which may

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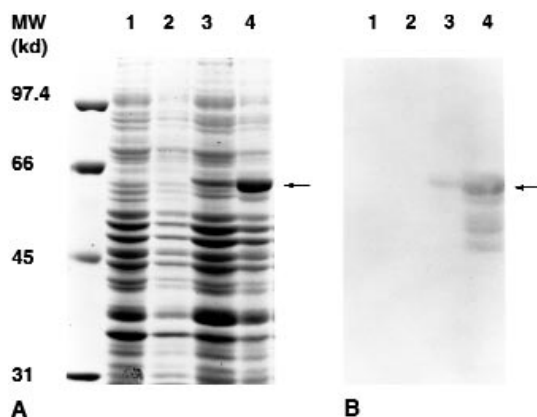


FIG 1. SDS-PAGE and IgE reactivity of sera from subjects with crustacean allergy against recombinant fusion proteins. **A**, Coomassie brilliant blue R-250 stain of *Escherichia coli* clone of uninduced pGEX 1 (lane 1) and IPTG-induced pGEX 1 (lane 2), uninduced Cha f 1 (lane 3), and IPTG-induced Cha f 1 (lane 4). Note presence of induced recombinant protein at 60 kD in lane 4. **B**, Identical gel, transferred onto nitrocellulose membrane and probed against sera from subjects with crustacean allergy for IgE reactivity. Note presence of strong reactive band at 60 kD (arrow) in induced Cha f 1 (lane 4). Weaker reactive band in lane 3 is result of IgE binding to baseline expression of recombinant Cha f 1 in uninduced cells. Normal control sera did not react (data not shown). Molecular weight in kilodaltons.

represent the snow crab tropomyosin. To further characterize and confirm the biochemical identity of the crab allergen at the molecular level, we report herein the isolation, expression, and primary structure determination of a cDNA coding for the major crab allergen.

METHODS

Sera

Sera from 10 patients with a well-documented history of type I hypersensitivity upon ingestion of crab were obtained from the sera bank at the Division of Rheumatology/Allergy and Clinical Immunology at University of California, Davis. These sera were shown to have IgE reactivity to multiple seafood including shrimp, lobster, and crab.¹⁵ Sera from 5 normal volunteers without any previous history of seafood allergy were used as controls.

Construction of cDNA library

Total RNA was isolated from the claw muscle of *Charybdis feriatius* by the guanidium isothiocyanate method with the use of the TRIzol reagent (Life Technologies, Gaithersburg, Md), with 0.5 g of muscle tissue in 5 mL of reagent. The cDNA library was then constructed with the CapFinder PCR cDNA Library Construction Kit (Clontech, Palo Alto, Calif). After being ligated into *EcoRI* ends of λ gt 11, the library was packaged in λ phage with the Gigapack III Gold Packaging Extract (Stratagene, La Jolla, Calif) as described.¹⁷

Screening of *Charybdis feriatius* cDNA library

A *Charybdis feriatius* cDNA library was screened with the use of methods as described.^{17,18} Briefly, an aliquot of the *Charybdis feriatius* cDNA library was plated on LB-agar by infecting *Escherichia coli* Y1090 at a density of 20,000 to 30,000 per plate. Plaques were transferred onto nitrocellulose filters. Thereafter, the filters were blocked with blotto (3% milk in PBS) and incubated with sera from patients with crab allergy (1:10 dilution) at 4°C for 8 to 10 hours. The filters were then washed 3 times with PBS containing 0.05% Tween 20 and then incubat-

ed with ¹²⁵I-labeled anti-human IgE (Sanofi-Pasteur Diagnostics, Ohaska, Minn) at 4°C for 8 to 10 hours. Filters were washed as before and exposed to x-ray film. Positive signals were purified to homogeneity.

Nucleotide sequence determination of cDNA

Phage DNA was isolated from the purified IgE-reactive λ gt 11 clone as described,¹⁸ and the cDNA insert was excised by *EcoRI* digestion. The cDNA was then gel purified on low-melting-temperature agarose and ligated into the *EcoRI* site of plasmid vector pSK- (Stratagene, La Jolla, Calif). After transformation, miniprep DNA was prepared from transformants to test for the correct size of insert. Plasmid DNA was chosen from a colony with the same size of cDNA insert as in the IgE-reactive λ gt11 clone. The nucleotide sequence of the cDNA was determined by cycle sequencing of the plasmid DNA with AmpliTaq DNA polymerase and dye-labeled terminators with the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Inc, Foster City, Calif) and sequencing primers T3 and T7 as well as primers TM3 (5'-ATGAAGCTGGA-GAAGGA-3'), TM4 (5'-GTAGCCAGACAGTTCG-3'), and L1 (5'-ACCTCGTCGTATTCCTGT-3'), which correspond to nucleotide No. 22-38, reverse and complementary sequence of nucleotides 843-827, and reverse and complementary sequence of nucleotides 485-467, respectively, derived from *Metapenaeus ensis* tropomyosin (Genbank Database, Accession No. U08008). Nucleotide sequences were analyzed with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems Inc) and further edited by the software Sequence Editor, version 1.0.3 (Applied Biosystems Inc).^{19,20}

Expression of cDNA

cDNA encoding the IgE-reactive protein was cloned into the *EcoRI* site of plasmid expression vectors pGEX²¹ with primer pairs 5'-GGAATTCATGGACGCCATCAAGAAG-3' (nucleotide sequence 1-18 of Cha f 1) and 5'-GGAATTCAACAAGTTCATCTCAAGGCC-3' (reverse and complementary sequence of nucleotides 783-763 of Cha f 1) as forward and reverse primers, respectively, to amplify the coding sequence of Cha f 1 cDNA. Restriction enzyme *EcoRI* sites were added to the 5' ends of both forward and reverse primers for cloning purposes. Briefly, a PCR was performed at 95°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute for 30 cycles, and the resulting PCR product was purified, digested with *EcoRI*, and ligated with *EcoRI*-digested pGEX vectors and transformed into *E. coli* DH5 α . Transformants were selected and a colony immunoassay performed. Briefly, transformants were picked onto nitrocellulose filters and the filters incubated on LB agar plates containing 25 μ g/mL ampicillin for 10 hours at 37°C and then induced by transferring the filter to LB agar plates containing 25 μ g/mL ampicillin plus 1 mmol/L IPTG. An identical set of nitrocellulose filters was grown on a master plate. After 4 hours of incubation at 37°C, cells were lysed by chloroform vapor and the filters incubated with blotto for blocking. After blocking, the filters were incubated with sera (n = 10) from patients with crustacean allergy (1:10 dilution) at 4°C for 8 to 10 hours. Sera from normal subjects (n = 5) and volunteers without seafood allergy (n = 5) were run in parallel as controls. The filters were then washed 3 times with PBS containing 0.05% Tween 20 and then incubated with ¹²⁵I-labeled anti-human IgE (Sanofi-Pasteur Diagnostics, Ohaska, Minn) at 4°C for 8 to 10 hours. The filters were washed as before and exposed to x-ray film for 48 hours. Positive clones were identified and then reconfirmed for the presence of inserts by miniprep DNA preparation and agarose gel electrophoresis.

Determination of molecular weight of expressed IgE-reactive crab protein by immunoblotting

Positive expression clones were grown in LB containing 25 μ g/mL ampicillin at 37°C overnight and then induced by 1 mmol/L IPTG for 4 hours at 37°C. The cell lysate was resolved on 8% SDS-

TABLE I. Amino acid composition of tropomyosins from various shellfish, fruitfly, and chicken*

Amino acid	Cha f 1	Met e 1	Pan s 1	Hom a 1	Fruit fly	Chicken
Alanine (A)	35	31	31	33	25	34
Cysteine (C)	0	1	0	0	3	1
Aspartic acid (D)	15	17	17	18	22	25
Glutamic acid (E)	53	55	55	55	51	54
Phenylalanine (F)	2	4	4	3	5	1
Glycine (G)	3	5	4	4	3	3
Histidine (H)	0	1	1	1	1	1
Isoleucine (I)	5	4	4	6	7	9
Lysine (K)	27	25	25	28	35	40
Leucine (L)	28	33	33	33	28	38
Methionine (M)	8	6	6	8	8	4
Asparagine (N)	15	18	16	16	13	6
Proline (P)	1	0	0	0	0	0
Glutamine (Q)	17	17	16	17	19	12
Arginine (R)	21	22	21	21	17	13
Serine (S)	11	15	14	15	14	17
Threonine (T)	9	9	10	9	14	10
Valine (V)	12	14	13	13	17	9
Tyrosine (Y)	2	4	4	4	2	7
Total	264	281	274	284	284	284

*Amino acid composition are from deduced amino acid sequence of Cha f 1 (this work), Met e 1,¹¹ Pan s 1,¹ Hom a 1, and Ha smT (*Homarus americanus* slow muscle tropomyosin),²⁴ fruit fly,³⁰ and chicken³¹ tropomyosin.

PAGE, which was then transferred onto a nitrocellulose filter as described.²² Sera from patients with crustacean allergy (1:10 dilution) were then used to probe the filters and IgE reactivity determined with the use of ¹²⁵I-labeled anti-human IgE (Sanofi-Pasteur Diagnostics, Ohaska, Minn). After washing and exposing to x-ray film, the position of positive band was located and compared with the molecular weight marker.

Purification of IgE-reactive *Charybdis feriatus* protein Cha f 1

A 500-mL overnight culture of a pGEX expressing clone of the IgE-reactive *Charybdis feriatus* protein was induced with 1 mmol/L IPTG, and the cells were harvested. The cells were suspended in 5 mL of lysis buffer (1% Triton X-100, 1% Tween 20, 10 mmol/L dithiothreitol) and sonicated. The cell lysate was then spun to pellet the debris, and the supernatant was added to glutathione agarose. After incubating for 2 hours on a rocker, the glutathione agarose was washed 3 times with PBST (PBS containing 1% Triton X-100, pH 7.3) and the bound protein eluted with 2 mL of 5 mmol/L reduced glutathione in 50 mmol/L Tris-HCl, pH 8.0. The purified protein was quantified and the purity determined by SDS-PAGE and immunoblotting as described. IgE reactivity from subjects with crustacean allergy, normal volunteers, and other subjects with allergy but without seafood allergy against recombinant Cha f 1 were tested by immunoblotting as described above.

Inhibition of IgE reactivity to *Charybdis feriatus* extract by recombinant Cha f 1

Charybdis feriatus claw muscle extract was prepared by homogenizing 0.5 g of the claw muscle in PBS, pH 7.2, with a polytron. Thereafter, the extract was mixed for 8 hours at 4°C and then centrifuged at 5000g for 15 minutes. The supernatant was collected and stored in small aliquots at -20°C until use. Sera (at 1:10 dilution in PBS containing 3% milk powder) from subjects with crustacean allergy were incubated for 8 to 12 hours at 4°C with 100 µg of recombinant Cha f 1 in a final volume of 1 mL. Sera were also incubated with 100 mg of recombinant glutathione S-transferase as con-

trol. The absorbed sera were then tested for their IgE reactivities against crab extract and recombinant Cha f 1 by immunoblotting.

Inhibition of IgE reactivity to *Cha f 1* by recombinant shrimp protein Met e 1 and Pan s 1

Sera from patients (1:10 dilution) with known IgE reactivity to crustaceans were incubated with Met e 1 and Pan s 1¹⁷ recombinant protein separately at 100 µg/mL with 3% milk in PBS at 4°C overnight on a rocker. Absorbed sera were analyzed for their reactivity to Cha f 1 by immunoblotting. Briefly, recombinant Cha f 1 was resolved on polyacrylamide gel, transferred to a nitrocellulose filter, and then incubated with sera absorbed with Met e 1 and Pan s 1 at 4°C for 8 to 10 hours. The filters were then washed 3 times with PBS containing 0.05% Tween 20 and then incubated with ¹²⁵I-labeled anti-human IgE (Sanofi-Pasteur Diagnostics, Ohaska, Minn) at 4°C for 8 to 10 hours, washed, and exposed to x-ray films. Sera absorbed with an irrelevant mitochondrial autoantigen BCOADC-E2²² were run in parallel as control. In addition, IgE reactivity against recombinant Met e 1, Pan s 1, Hom a 1, and an irrelevant control protein BCOADC-E2 were run in parallel by immunoblotting.

RESULTS

Identification of an IgE-reactive clone from *Charybdis feriatus* cDNA library

A cDNA library was constructed from *Charybdis feriatus* claw muscle RNA. The unamplified library has 1.94×10^6 pfu primary recombinant clones. An IgE-reactive clone was identified by screening the cDNA library with sera from patients with crustacean hypersensitivity. The clone was purified to homogeneity and designated Cha f 1 according to the nomenclature proposed by Marsh et al.²³ Cha f 1 reacted to sera from patients with crustacean allergy but not to sera from normal volunteers or sera from patients with other allergies. Digestion of purified phage DNA with *Eco*RI gave a 1-kb cDNA fragment on agarose gel.

	1				50
Cha f I	MDAIKKKMQA	MKLEKDNAMD	RADTLEQQNK	EANLRAEKTE	EEIRATQKKM
Pan s I	-----	MKLEKDNAMD	RADTLEQQNK	EANLRAEKAE	EEVHNLQKRM
Hom a I	MDAIKKKMQA	MKLEKDNAMD	RADTLEQQNK	EANLRAEKSE	EEVHNLQKRM
Met e I	---EFCGRQA	MKLEKDNAMD	RADTLEQQNK	EANNRAEKSE	EEVHNLQKRM
	51				100
Cha f I	QQVENELDQA	QEQLSAANTK	LDEKEKALQN	AEGEVAALNR	RIQLPEEDLE
Pan s I	QQLENDLDQV	QESLLKANTQ	LEEKDKALSN	AEGEVAALNR	RIQLLEEDLE
Hom a I	QQLENDLDQV	QESLLKANTQ	LEEKDKALSN	AEGEVAALNR	RIQLLEEDLE
Met e I	QQLENDLDQV	QESLLKANNO	LVEKDKALSN	AEGEVAALNR	RIQLLEEDLE
	101				150
Cha f I	RSEERLNTAT	TKLAEASQAA	DESERMKVL	ENRSLSDEER	MDALENQLKE
Pan s I	RSEERLNTAT	TKLAEASQAA	DESERMKVL	ENRSLSDEER	MDALENQLKE
Hom a I	RSEERLNTAT	TKLAEASQAA	DESERMKVL	ENRSLSDEER	MDALENQLKE
Met e I	RSEERLNTAT	TKLAEASQAA	DESERMKVL	ENRSLSDEER	MDALENQLKE
	151				200
Cha f I	ARFLAEADR	KYDEVARKLA	MVEADLERAE	ERAESGESKI	VELEEELRVV
Pan s I	ARFLAEADR	KYDEVARKLA	MVEADLERAE	ERAETGESKI	VELEEELRVV
Hom a I	ARFLAEADR	KYDEVARKLA	MVEADLERAE	ERAETGESKI	VELEEELRVV
Met e I	ARFLAEADR	KYDEVARKLA	MVEADLERAE	ERAETGESKI	VELEEELRVV
	201				250
Cha f I	GNNLKSLEVS	EEKANQREET	YKEQIKTLAN	KLKAAEARAE	FAERSVQKLQ
Pan s I	GNNLKSLEVS	EEKANQREEA	YKEQIKTLTN	KLKAAEARAE	FAERSVQKLQ
Hom a I	GNNLKSLEVS	EEKANQREEA	YKEQIKTLAN	KLKAAEARAE	FAERSVQKLQ
Met e I	GNNLKSLEVS	EEKANQREEA	YKEQIKTLTN	KLKAAEARAE	FAERSVQKLQ
	251				284
Cha f I	KEVDRLEDEL	VNEK----	-----		
Pan s I	KEVDRLEDEL	VNEKEKYKSI	TDELDQTFSE	LSGY	
Hom a I	KEVDRLEDEL	VNEKEKYKSI	TDELDQTFSE	LSGY	
Met e I	KEVDRLEDEL	VNEKEKYKSI	TDELDQTFSE	LSGY	

Comparison of Amino Acid Sequence of Cha f 1, Pan s 1, Hom a 1 and Met e 1. Amino Acid different from that of Met e 1 are underlined.

FIG 2. Amino acid comparison of Cha f 1 with other crustacean tropomyosin Pan s 1, Hom a 1, Met e 1.

Expression and specific reactivity of Cha f 1

The Cha f 1 cDNA was subcloned into frame-shifted plasmid expression vectors pGEX²¹ in *E. coli* DH5 α . Colony immunoassays showed that the protein was expressed in pGEX 1 but not in pGEX 2 and pGEX 3. Sera from normal volunteers as well as from patients with other allergies did not react. The molecular weight of the expressed protein was determined by SDS-PAGE. Comparison of cell lysates of Cha f 1 in pGEX 1 and pGEX 1 plasmid control by SDS-PAGE showed the presence of a 60-kd fusion protein band in pGEX 1 Cha f 1 but not in pGEX 1 control. Immunoblotting of the cell lysate demonstrated that the 60-kd band is reactive to IgE from patients with crustacean allergy. The fusion protein was further purified by glutathione agarose; the purified fusion protein demonstrated a pure protein band of 60-kd and retained its IgE reactivity with sera from patients sensitive to crab. Because the glutathione S-transferase has a molecular weight of 26 kd, the molecular weight of Cha f 1 was estimated to be 34 kd by SDS-PAGE (Fig 1).

Sequence determination of Cha f 1

The deduced amino acid sequence of Cha f 1 is shown in Fig 2. Nucleotide sequence analysis of Cha f 1 cDNA (Genbank accession No. AF 061783) showed an open reading frame of 264 amino acids, followed by a long poly A tail after nucleotide 792. Thus even though Cha f 1 is shorter than Met e 1 by 20 amino acid residues (Fig 2), Cha f 1 is unlikely to be a truncated form of crab

tropomyosin. The amino acid composition of Cha f 1 is shown in Table I. The protein is rich in alanine, glutamic acid, lysine, leucine, and arginine and does not contain cysteine, histidine, and tryptophan. Genbank data search of Cha f 1 reveals that the protein is 89.7% identical with the shrimp tropomyosin Met e 1⁹ and is 95.8% identical with the slow muscle tropomyosin of lobster²⁴ (Table II).

Specific inhibition of IgE reactivity to crab muscle by Cha f 1

Sera from patients with crustacean allergies lost their IgE reactivity to crab lysate when they were preincubated with recombinant Cha f 1 protein, whereas when sera were absorbed by an irrelevant control protein, the BCOADC-E2 still retained their IgE reactivity to crab lysate (Fig 3, A).

Specific inhibition of IgE reactivity to Cha f 1 by Met e 1 and Pan s 1

After absorption with recombinant proteins of Met e 1 and Pan s 1, sera from subjects with crustacean allergy lost their IgE reactivity to Cha f 1, whereas unabsorbed sera and sera absorbed with an irrelevant protein BCOADC-E2 retained the IgE reactivity to Cha f 1 (Fig 3, B).

DISCUSSION

Hypersensitive reactions to shellfish such as shrimp and crab are one of the most common causes of adverse food reactions among hypersensitive individuals. In the United States, it is estimated more than 250,000 individuals are at risk of having clinically significant immuno-

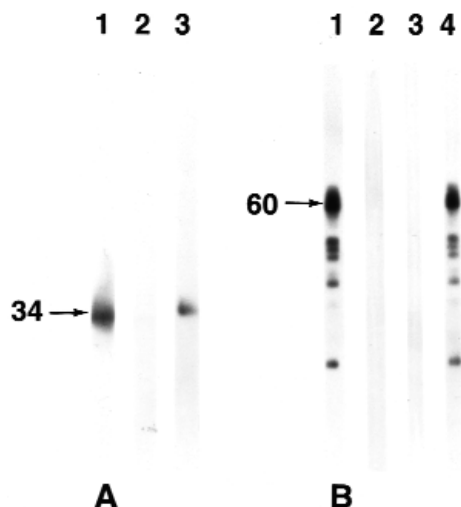


FIG 3. A, Specific inhibition of IgE reactivity against *Charybdis feriatus* muscle protein by Cha f 1. Note presence of major reactive band with unabsorbed sera at 34 kD (lane 1) and loss of reactivity when sera were absorbed by recombinant proteins of Cha f 1 (lane 2). Sera absorbed by irrelevant control recombinant protein BCOADC-E2 did not inhibit reactivity (lane 3). **B,** Specific inhibition of IgE reactivity to Cha f 1 by recombinant protein Met e 1 and Pan s 1. Note presence of 60-kD band in unabsorbed lane (lane 1) and loss of 60-kD band when the sera were absorbed by Met e 1 (lane 2) and Pan s 1 (lane 3), whereas absorption by an irrelevant control protein did not remove IgE reactivity against recombinant Cha f 1 (lane 4). Lower molecular weight reactive bands probably correspond to IgE reactivity to degraded Cha f 1 recombinant proteins.

logic responses upon ingestion of shellfish.² Crabs and shrimp are the most commonly consumed shellfish. In individuals allergic to shrimp, specific IgE antibody reactivity to shellfish has been demonstrated by prick skin test and the radioallergosorbent test.^{5,6,25} These specific IgE antibodies probably account for immediate hypersensitive responses upon exposure or ingestion of shellfish. To date, commercially available shellfish allergen preparations are prepared from boiled extracts prepared from multiple species or genera²⁶ and thus their reproducibility and reliability have not been adequately evaluated and are often highly variable. The importance of obtaining purified and characterized shellfish allergens for improving the current diagnostic and therapeutic agents have been discussed.⁴ Several studies on methods in preparing and characterizing shrimp extracts have suggested the major IgE reactive component in shrimp is a 34- to 38-kD heat-stable protein of pI 4.5.^{7,27} It was not until recently that the major heat-stable shrimp allergen was identified as tropomyosin and its cDNA sequence determined.^{9,11}

It has been reported that individuals with a history of shrimp allergy also demonstrate allergic reactions to other species of crustaceans.⁵ With the use of crossed immunoelectrophoresis, the presence of shared antigenic determinants were shown among various shellfish including white shrimp, blue crab, crawfish, and lobster.²⁸ However, the biochemical identity of this cross-reactive molecule was unknown. Recently, our laboratory has studied

TABLE II. Amino acid sequence identity of crustacean, fruit fly, and chicken tropomyosin*

% Identity	Cha f 1	Met e 1	Pan s 1	Hom a 1	Ha smT	Fruit fly	Chicken
Cha f 1	100						
Met e 1	89.7	100					
Pan s 1	91.4	98.2	100				
Hom a 1	92.4	94.7	97.5	100			
Ha smT	95.8	89.3	92.3	94.7	100		
Fruit fly	68.6	65.5	66.8	67.9	68.2	100	
Chicken	60.3	46.4	47.6	48.2	47.9	39.7	100

*Expressed to the nearest 1 decimal space in % according to deduced amino acid sequence of Cha f 1 (this work), Met e 1,¹¹ Pan s 1,¹⁷ Hom a 1 and Ha smT (*Homarus americanus* slow muscle tropomyosin),²⁴ fruit fly,³⁰ and chicken³¹ tropomyosin.

TABLE III. IgE reactivity against recombinant shellfish allergens*

Groups	Recombinant proteins				
	Cha f 1	Met e 1	Pan s 1	Hom a 1	BCOADC-E2
Subjects with crab allergy (n = 10)	10/10†	10/10	10/10	10/10	0/10
Other subjects with allergy (n = 5)	0/5	0/5	0/5	0/5	0/5
Normal volunteers (n = 5)	0/5	0/5	0/5	0/5	0/5*

*Sera were tested for their IgE reactivity against recombinant proteins of Cha f 1, Met e 1, Pan s 1, Hom a 1, and an irrelevant protein control BCOADC-E2 by immunoblotting as described in Methods.

†Expressed as number reactive/total sera tested.

the cross-reactivity of crustacean allergens among shrimp, lobster, and crab.¹⁵ By specific absorption studies with recombinant shrimp tropomyosin, the protein was shown to share immunodominant IgE epitopes, reflecting that tropomyosin is also the predominant allergen common to crustaceans. To further illustrate this at the molecular level, we constructed a cDNA library from *Charybdis feriatus* and screened this cDNA library with sera from patients with clinical history of crab allergy.

Charybdis feriatus is a common crab species consumed as seafood throughout its geographical range extending from Japan to the east coast of Africa.²⁹ Many other species of the same genus are also of commercial importance. This genus belongs to the family Portunidae (swimming crabs), which includes most of the economic crab species, such as the blue crab *Callinectes sapidus* in the Atlantic coast of North and South America, the mud crab *Scylla serrata* in the Indo-West Pacific, and various *Portunus* species throughout the world.

Expression of the Cha f 1 cDNA produces a recombinant protein recognized by sera IgE from patients with crustacean allergy, indicating that the recombinant protein is biologically active in expressing its IgE determinants. Sera

from subjects with crustacean allergy reacted not only to Cha f 1 recombinant protein but also to recombinant proteins of other shellfish allergens including Met e 1, Pan s 1, and Hom a 1 (Table III).^{30,31} Moreover, preincubation of the sera with Cha f 1 protein inhibited the sera IgE reactivity to crab lysate by immunoblotting, suggesting that the Cha f 1 recombinant protein contains all the IgE epitopes recognized by sera from patients with crustacean allergy. Furthermore, the inhibition of IgE reactivity to Cha f 1 by Met e 1 and Pan s 1 demonstrated that Cha f 1 shares the common IgE epitope with other shellfish tropomyosin (Met e 1, Pan s 1) and that Cha f 1 is the major crab allergen.

Nucleotide sequence analysis of Cha f 1 shows that the major IgE-reactive protein in crab is tropomyosin. Interestingly, Cha f 1 shares extensive similarity in amino acid composition and peptide sequence identity with other forms of tropomyosin (Table II), most significantly with the *Homarus americanus* slow muscle tropomyosin at 95.8% and lesser with the *Drosophila* tropomyosin at 60.3%. Interestingly, Cha f 1 is also significantly homologous with the *H. americanus* fast muscle tropomyosin Hom a 1 at 92.4% and *Panulirus stimpsoni* tropomyosin Pan s 1 at 91.4%, respectively. Altogether, our immunologic data and sequence analysis clearly show that Cha f 1 is the crab tropomyosin. Furthermore, the fact that the *Charybdis feriatus* cDNA library is constructed from the claw muscle and the 95.8% sequence identity of Cha f 1 with the *H. americanus* slow muscle tropomyosin indicate that Cha f 1 corresponds to the crab slow muscle tropomyosin. On the other hand, the other shellfish allergens (Met e 1, Pan s 1, and Hom a 1) that we have previously isolated from the tail muscle of shrimp and lobster^{11,17} possibly represent the fast muscle tropomyosin. The isolation and expression of crab tropomyosin cDNA offers the possibility of obtaining well-defined homogeneous crab allergen as reagents for development of research, clinical diagnosis, and therapeutic applications.

Final note

New allergen data on Cha f 1 have been submitted to the WHO/IUIS Allergen Nomenclature Subcommittee.

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REFERENCES

1. Leung PSC, Chu KH. Molecular and immunological characterization of shellfish allergens. *Front Biosci* 1998;3:306-12.
2. Henriksen O. Scientist net major shrimp allergen. National Center for Research Resources Reporter. 1994;XVIII:10-1.
3. Daul CB, Slatery M, Lehrer SB. Shared antigenic/allergenic epitopes between shrimp *Pen a 1* and fruitfly extract [abstract]. *J Allergy Clin Immunol* 1993;91:341.
4. Daul CB, Morgan JE, Lehrer SB. Hypersensitivity reactions to crustacea and mollusks. *Clin Rev Allergy* 1993;11:201-22.
5. Waring NP, Daul CB, Deshaza RD, McCants ML, Lehrer SB. Hypersensitivity reactions to ingested Crustacea: clinical evaluation and diagnostic studies in shrimp-sensitive individuals. *J Allergy Clin Immunol* 1985;76:440-5.
6. Daul CB, Morgan JE, Waring NP, McCants ML, Hughes J, Lehrer SB. Immunologic evaluation of shrimp allergic individuals. *J Allergy Clin Immunol*. 1987;80:716-22.
7. Hoffman DR, Day ED, Miller JS. The major heat stable allergen of shrimp. *Ann Allergy* 1981;47:17-22.
8. Nagpal S, Metcalfe DD, Subba Rao PV. Identification of a shrimp derived allergen as tRNA. *J Immunol* 1987;138:4169-74.
9. Shanti KN, Martin BM, Nagpal S, Metcalfe DD, Subba Rao PV. Identification of tropomyosin as the major shrimp allergen and characterization of its IgE binding epitopes. *J Immunol* 1993;151:5354-63.
10. Daul CB, Slatery M, Reese G, Lehrer SB. Identification of the major brown shrimp (*Penaeus aztecus*) allergen as the muscle protein tropomyosin. *Int Arch Allergy Appl Immunol* 1994;105:49-55.
11. Leung PSC, Chu KH, Chow WK, Ansari A, Bandea CI, Kwan HS, Nagy SM, Gershwin ME. Cloning, expression, and primary structure of *Metapenaeus ensis* tropomyosin, the major heat-stable shrimp allergen. *J Allergy Clin Immunol* 1994;94:882-90.
12. Hanke PD, Storti RV. Nucleotide sequence of a cDNA clone encoding a *Drosophila* muscle tropomyosin II isoform. *Gene* 1986;45:211-4.
13. Ruiz-Opazo N, Weinbergere J, Nadal-Ginard B. Comparison of α -tropomyosin sequences from smooth and striated muscle. *Nature* 1985;315:67-70.
14. Reese G, Jeoung B-J, Daul CB, Lehrer SB. Characterization of recombinant shrimp allergen *Pen a 1* (Tropomyosin). *Int Arch Allergy Immunol* 1997;113:240-2.
15. Leung PSC, Chow WK, Duffey S, Kwan HS, Gershwin ME, Chu KH. IgE reactivity against a cross-reactive allergen in crustacea and mollusca: evidence for tropomyosin as the common allergen. *J Allergy Clin Immunol* 1996;98:954-61.
16. Hefle SL, Bush RK, Lehrer SB, Malo J-L, Cartier A. Snow crab allergy: identification of IgE-binding proteins [abstract]. *J Allergy Clin Immunol* 1995;95:322.
17. Leung PSC, Chen YC, Mykles DL, Chow WK, Li CP, Chu KH. Molecular identification of the lobster muscle protein tropomyosin as a seafood allergen. *Mar Mol Biol Biotech* 1998;7:12-20.
18. Manitis T, Fritsch EF, Sambrook J. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory; 1982.
19. Horton JH, Hagen MD, Ko MS. Optimized conditions for cycle sequencing of PCR products. *PCR Methods Appl* 1994;3:359-60.
20. Daniel SE. Preparation and direct automated cycle sequencing of PCR products. *Methods Mol Biol* 1996;65:155-61.
21. Smith DB, Johnson KS. Single step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene* 1988;76:31-40.
22. Leung PSC, Chuang DT, Wynn RM, Cha S, Danner DJ, Ansari A, et al. Autoantibodies to BCOADC-E2 in patients with primary biliary cirrhosis recognize a conformational epitope. *Hepatology* 1995;22:505-13.
23. Marsh DG, Goodfriend L, King TP, Lowenstein H, Platts M, Ills TAE. Allergen nomenclature. *Int Arch Allergy Appl Immunol* 1988;85:194-200.
24. Mykles DL, Cotton JLS, Taniguchi H, Sano KI, Maeda Y. Cloning of tropomyosins from lobster (*Homarus americanus*) striated muscles: fast and slow forms may be generated from the same transcript. *J Muscle Res Cell Motil* 1998;19:105-16.
25. Morgan JE, Daul CB, Hughes J, McCants M, Lehrer SB. Food specific skin-test reactivity in atopic subjects. *Clin Exp Allergy* 1989;19:431-5.
26. Cartier A, Malo JL, Ghezzi H, McCants M, Lehrer SB. IgE sensitization in snow crab-processing workers. *J Allergy Clin Immunol* 1986;78:344-8.
27. Nagpal S, Rajappa L, Metcalfe DD, Subba Rao PV. Isolation and characterization of heat-stable allergens from shrimp (*Penaeus indicus*). *J Allergy Clin Immunol* 1989;83:26-36.
28. Lehrer SB. The complex nature of food antigens: studies of cross-reacting crustacea allergens. *Ann Allergy* 1986;57:267-72.
29. Wee PC, Ng PKL. Swimming crabs of the genera *Charybdis de Hann*, 1833, and *Thalamita Latreille*, 1829 (Crustacea: Decapoda: Brachyura: Portunidae) from Peninsular Malaysia and Singapore. *affles Bull Zool*. 1995;Suppl 1:128.
30. Basi GS, Storti RV. Structure and DNA sequence of the tropomyosin I gene from *Drosophila melanogaster*. *J Biol Chem* 1985;261:817-27.
31. Goodling C, Reinach FC, Macleod AR. Complete nucleotide sequence of the fast-twitch isoform of chicken skeletal muscle α -tropomyosin. *Nucleic Acids Res* 1987;15:8105.