

Molecular identification of the lobster muscle protein tropomyosin as a seafood allergen

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Abstract

Crustaceans are a major cause of seafood allergy. Recent studies have identified tropomyosin as the major allergen in shrimp. However, such data are lacking in other crustaceans. In the present study lobster allergens were identified and characterized by molecular cloning, sequencing, and expression. An IgE-reactive complementary DNA clone of 2 kilobase pairs (kb) was identified by screening an expression library of the spiny lobster *Panulirus stimpsoni* using sera from subjects with crustacean allergy. Expression and sequencing of this clone showed that it has an opening reading frame of 274 amino acids, coding for a 34-kDa protein designated as *Pan s I*. In addition, we expressed the fast muscle tropomyosin from the American lobster *Homarus americanus* and found that this protein, coined *Hom a I*, was also recognized by IgE from patients with crustacean allergies. The deduced amino acid sequences of *Pan s I* and *Hom a I*, which are the first identified lobster allergens, show significant homology to shrimp tropomyosin. Sera from subjects with crustacean allergies, when pre-

absorbed with recombinant proteins *Pan s I* or *Hom a I*, lost their IgE reactivity to muscle extract of *P. stimpsoni* and *H. americanus*. Preincubation of crustacean allergy sera with the recombinant shrimp tropomyosin *Met e I* also removed their IgE reactivity to lobster muscle extracts. The results suggest that patients with allergic reactions to crustaceans have common and possibly cross-reactive IgE-reactive epitopes in lobster and shrimp.

Introduction

IgE-mediated hypersensitive reactions to ingestion of shellfish are among the most serious forms of food allergies (Daul et al., 1993a; O'Neil and Lehrer, 1995). It is estimated that at least 250,000 people in the United States have a high risk of developing allergic reactions to the ingestion of seafood products (Lehrer et al., 1992; Henriksen, 1994). Sensitized individuals can develop urticaria, angioedema, laryngospasm, asthma, and life-threatening anaphylaxis reactions (Waring et al., 1985; Daul et al., 1987). Although crustaceans have been reported to be one of the more common causes of hypersensitivity reactions to foods, reports on the characterization and identification of shellfish allergens are primarily limited to the penaeid shrimp.

Hoffman et al. (1987) demonstrated the presence of heat-stable and heat-labile allergens in shrimp. The heat-stable allergen, namely antigen II, was identified as a 38-kDa protein. Nagpal et al. (1989) reported the isolation of two heat-stable reactive shrimp allergens, designated as Sa-I and Sa-II. Sa-II with a molecular weight of 34 kDa was identified to be a peptide of 301 amino acid residues. A comparison of amino acid composition suggests that antigen II and Sa-II are the same allergenic material, representing the major shrimp allergen (Daul et al., 1993a). It was not until recently that this allergen was identified as the muscle protein tropomyosin (Shanti et al., 1993; Daul et al., 1994; Leung et al., 1994).

The cDNA and amino acid sequence of the major

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shrimp allergen *Met e I*, derived from *Metapenaeus ensis*, has been reported (Leung et al., 1994). The nucleotide sequence of this cDNA clone reveals an open reading frame of 281 amino acid residues, coding for a protein of 34 kDa. Comparison of the *Met e I* amino acid sequence with the GenBank database shows that *Met e I* is homologous to multiple isoforms of tropomyosin (Ruiz-Opazo et al., 1985; Hanke and Storti, 1986). Shanti et al. (1993) also demonstrated an 86% homology between Sa-II and tropomyosin by comparing amino acid sequences of different peptides generated by proteolysis of Sa-II. Thus, it is now well established that tropomyosin is the major shrimp allergen.

Although IgE cross-reactivity between various crustaceans has been reported, the molecular identity of the cross-reactive allergens has not been well studied (Halmenpuro et al., 1987; Musmand et al., 1993). Recently, using recombinant protein of shrimp tropomyosin *Met e I*, our laboratory has shown by immunoblotting that *Met e I* can absorb all the IgE reactivity of crustacean allergy sera against a number of crustacean and mollusk proteins, demonstrating that tropomyosin is a common allergen in these shellfish (Leung et al., 1996). Owing to the increasing worldwide demand and consumption of seafood (Moody et al., 1993) including crustaceans, it is expected that a considerable number of patients with shrimp allergy will also develop an allergic reaction after the ingestion of other crustaceans such as lobster, crab, and crayfish. To further characterize the crustacean allergens at the molecular level, we have isolated an IgE-reactive protein from a cDNA library of the spiny lobster *Panulirus stimpsoni* and identified the protein as the lobster tropomyosin coined *Pan s I*. Furthermore, comparison of amino acid sequence and specific IgE immunoreactivity of *Pan s I* with tropomyosins from the American lobster *Homarus americanus* and the shrimp tropomyosin *Met e I* demonstrates that the lobster tropomyosin is a major lobster allergen.

Results

Identification of an IgE-reactive clone from *Panulirus stimpsoni* cDNA library

A cDNA library was constructed from *P. stimpsoni* muscle RNA. The unamplified library has 1.94×10^6 PFU primary recombinant clones, which correspond to 95.7% of the library. An IgE-reactive clone was identified by screening the cDNA library with sera from patients with hypersensitivity on inges-

tion of crustaceans. The clone was purified to homogeneity and designated *Pan s I* according to the nomenclature proposed by Marsh et al. (1989). *Pan s I* 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 *EcoRI* gave a 2-kb cDNA fragment on agarose gel.

Expression of *Pan s I*

The *Pan s I* cDNA was subcloned into frame-shifted plasmid expression vector pGEX (Smith and Johnson, 1988). Results of colony immunoassays showed that the protein was expressed in pGEX 1 but not in pGEX 2 and pGEX 3. Sera from normal volunteers did not react. The molecular weight of the expressed protein was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Comparison of cell lysates of *Pan s I* in pGEX 1 and pGEX 1 plasmid control by SDS-PAGE showed the presence of a 60-kDa fusion protein band in pGEX 1 with *Pan s I*, but not in pGEX 1 control. Immunoblotting of the cell lysate demonstrated that the 60-kDa band is reactive to IgE from patients with crustacean allergy (Figure 1A). This 60-kDa recombinant protein showed specific reactivity to IgE antibodies from patients with crustacean allergy, whereas sera from normal volunteers did not react (Figure 1B). Since the glutathione S–transferase has a molecular weight of 26 kDa, the molecular weight of *Pan s I* is estimated to be 34 kDa by SDS-PAGE.

Nucleotide sequence determination of *Pan s I*

Nucleotide sequence analysis of *Pan s I* cDNA showed an open reading frame of 274 amino acids (Figure 2). The amino acid composition of *Pan s I* is shown in Table 1. The protein is rich in alanine, glutamic acid, lysine, leucine, and arginine, but lacks cysteine, proline, and tryptophan. A GenBank data search of *Pan s I* revealed that the protein is 98.2% identical with *Met e I* (Figure 2).

Expression of *H. americanus* tropomyosin

The cDNA coding for *H. americanus* fast muscle tropomyosin was amplified by polymerase chain reaction (PCR) from a cDNA clone of the protein (Mykles et al., 1997). The amplified DNA was ligated into expression plasmid vector pGEX (Pharmacia, Piscataway, N.J.) and transformed into *Escherichia coli* XL1. Transformants were screened for IgE reactivity with sera from subjects with crustacean allergies. SDS-PAGE analysis of immunoreac-

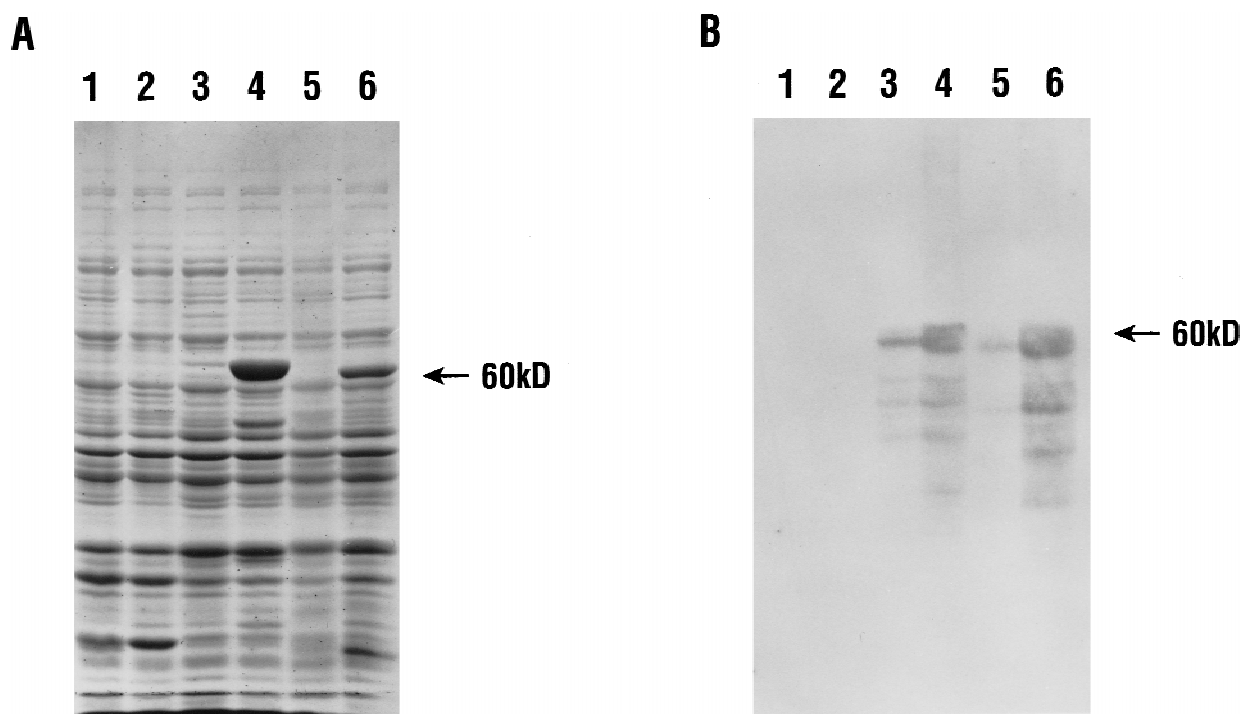


Figure 1. SDS-PAGE and IgE reactivity of sera from subjects with crustacean allergy against recombinant fusion proteins. (A) Coomassie brilliant blue R-250 stain of *E. coli* clone of uninduced pGEX 1 (lane 1) and IPTG-induced pGEX 1 (lane 2), uninduced *Pan s I* (lane 3), and IPTG-induced *Pan s I* (lane 4), uninduced *Hom a I* (lane 5), and induced *Hom a I* (lane 6). Note the presence of a recombinant protein at 60 kDa in lanes 4 and 6. (B) Identical gel, transferred onto nitrocellulose membrane and probed against sera from subjects with crustacean allergy for IgE reactivity. Note the strong reactive band at 60 kDa in the induced *Pan s I* (lane 4) and *Hom a I* (lane 6) the weaker band of uninduced *Pan s I* and *Hom a I* in lanes 3 and 5, respectively. The low molecular weight reactive bands are most likely due to reactivity to breakdown products of the 60-kDa band. Normal control sera did not react (data not shown).

tive colonies showed a prominent fusion protein band at 60 kDa consisting of the 26-kDa glutathione S-transferase fused to the lobster tropomyosin. IgE reactivity against this 60-kDa band was studied. Sera from patients with crustacean allergy reacted to this 60-kDa band by immunoblotting whereas sera from normal subjects did not react (Figure 1). The recombinant *H. americanus* tropomyosin was designated as *Hom a I*.

Specific inhibition of IgE reactivity by recombinant tropomyosin

The two recombinant lobster tropomyosins, *Pan s I* and *Hom a I*, inhibited IgE reactivity of sera from allergic subjects against recombinant as well as native lobster tropomyosin. Sera from all 10 subjects with crustacean allergy lost their IgE reactivity to lobster extracts when they were preincubated with recombinant *Pan s I*, *Hom a I*, or *Met e I*. Moreover, sera preincubated with *Met e I* did not react with

recombinant *Pan s I* or *Hom a I*, suggesting that similar, if not identical, epitopes were recognized between these proteins (Figure 3).

Amino acid composition and sequence comparison of Pan s I and Hom a I

The amino acid compositions of *Pan s I* and *Hom a I* are very similar. Both proteins are rich in glutamic acid, leucine, alanine, lysine, and arginine. Interestingly, these proteins do not have any cysteine, tryptophan, and proline. *Pan s I* and *Hom a I* each have an open reading frame of 274 and 284 amino acid residues, respectively, and they are 97.5% identical in amino acid sequences (Tables 1 and 2). Their amino acid differences are underlined in Figure 2.

Discussion

Crustaceans have long been known as a common cause of allergic reactions to ingestion of seafood.

	1				50
<u>Pan s I</u>	-----	MKLEKDNAMD	RADTLEQQNK	EANIRAEKAE	EEVHNLQKRM
<u>Met e I</u>	---EFCGRQA	MKLEKDNAMD	RADTLEQQNK	EANNRAEKSE	EEVHNLQKRM
<u>Hom a I</u>	MDAIKKMQA	MKLEKDNAMD	RADTELOQNK	EANIRAEKSE	EEVHNLQKRM
Ha sTm	MDAIKKMQA	MKLEKDNAMD	RADTELOQNK	EANIRAEKTE	EEIRITHKKM
	51				100
<u>Pan s I</u>	QQLENDLDQV	QESLLKANTQ	LEEKDKALSN	AEGEVAALNR	RIQLLEEDLE
<u>Met e I</u>	QQLENDLDQV	QESLLKANNQ	LYEKDKALSN	AEGEVAALNR	RIQLLEEDLE
<u>Hom a I</u>	QQLENDLDQV	QESLLKANTQ	LEEKDKALSN	AEGEVAALNR	RIQLLEEDLE
Ha sTm	QQVENELDQV	QEQLSLANTK	LEEKEKALQN	AEGEVAALNR	RIQLLEEDLE
	101				150
<u>Pan s I</u>	RSEERLNTAT	TKLAEASQAA	DESERMKVL	ENRSLSDDEER	MDALENQLKE
<u>Met e I</u>	RSEERLNTAT	TKLAEASQAA	DESERMKVL	ENRSLSDDEER	MDALENQLKE
<u>Hom a I</u>	RSEERLNTAT	TKLAEASQAA	DESERMKVL	ENRSLSDDEER	MDALENQLKE
Ha sTm	RSEERLNTAT	TKLAEASQAA	DESERMKVL	ENRSLSDDEER	MDALENQLKE
	151				200
<u>Pan s I</u>	ARFLAEEADR	KYDEVARKLA	MVEADLERAE	ERAETGESKF	VELEEEELRVV
<u>Met e I</u>	ARFLAEEADR	KYDEVARKLA	MVEADLERAE	ERAETGESKI	VELEEEELRVV
<u>Hom a I</u>	ARFLAEEADR	KYDEVARKLA	MVEADLERAE	ERAETGESKI	VELEEEELRVV
Ha sTm	ARFLAEEADR	KYDEVARKLA	MVEADLERAE	ERAETGESKI	VELEEEELRVV
	201				250
<u>Pan s I</u>	GNNLKSLEVS	EEKANQREEA	YKEQIKTLTN	KLKAAEARAE	FAERSVQKLQ
<u>Met e I</u>	GNNLKSLEVS	EEKANQREEA	YKEQIKTLTN	KLKAAEARAE	FAERSVQKLQ
<u>Hom a I</u>	GNNLKSLEVS	EEKANQREEA	YKEQIKTLAN	KLKAAEARAE	FAERSVQKLQ
Ha sTm	GNNLKSLEVS	EEKANQREEA	YKEQIKTLAN	KLKAAEARAE	FAERSVQKLQ
	251				284
<u>Pan s I</u>	KEVDRLEDEL	VNEKEKYKSI	TDELDTQTFSE	LSGY	
<u>Met e I</u>	KEVDRLEDEL	VNEKEKYKSI	TDELDTQTFSE	LSGY	
<u>Hom a I</u>	KEVDRLEDEL	VNEKEKYKSI	TDELDTQTFSE	LSGY	
Ha sTm	KEVDRLEDEL	VNEKEKYKSI	TDELDTQTFSE	LSGY	

Figure 2. Comparison of deduced amino acid sequence of fast muscle tropomyosin from *Panulirus stimpsoni* (GenBank accession number AF30063), *Metapeanaeus ensis*, *Homarus americanus*, and slow muscle tropomyosin of *Homarus americanus*. Amino acid residues different from that of *Pan s I* are underlined.

Persons sensitive to crustaceans commonly develop the symptoms of type I IgE-mediated hypersensitivity, such as urticaria, angioedema, pruritus, and anaphylaxis (Daul et al., 1993b; O'Neil and Lehrer, 1995). Unfortunately, many sensitized individuals can develop allergy to other crustaceans and seafood without previous exposure. Moreover, allergens from shrimp, crab, lobster, and crayfish have been demonstrated to be cross-reactive to one another by skin test and immunoassay (Daul et al., 1993b; O'Neil and Lehrer, 1995). Cross-reactivity has also been reported between crustaceans and other animals including cockroach (O'Neil et al., 1985), oyster (Lehrer and McCants, 1987), caddis fly (Koshte et al., 1989), chironomid larvae (Eriksson et al., 1989), and mite (Witteaman et al., 1994). Therefore, there may exist common allergens that share similar or identical epitopes among crustacean species and between arthropods and mollusks.

Identification of shellfish allergens is therefore important in elucidating the underlying mechanism of the hypersensitivity reactions to ingestion of seafood. Earlier identification of crustacean allergens has been focused on shrimp (Hoffman et al.,

1987; Nagpal et al., 1989). Recently, the major heat-stable shrimp allergen has been identified as the muscle protein tropomyosin (Shanti et al., 1993; Daul et al., 1994). The major heat-stable shrimp allergen *Met e I* has been cloned, sequenced, and expressed (Leung et al., 1994). The nucleotide sequence of *Met e I* shows a high homology to that of *Drosophila* tropomyosin. Recent studies suggest that tropomyosin is not the only shrimp allergen but appears to be the major allergen common to crustaceans and mollusks (Leung et al., 1996).

In this study, we have investigated the IgE reactivity of crustacean-sensitive individuals with lobsters *P. stimpsoni* and *H. americanus* at the molecular level. These two species are members of the family Palinuridae (spiny lobsters) and family Nephropidae (clawed lobsters), respectively, which represent the two common groups of edible lobsters. *Homarus americanus* constitutes important fisheries in the United States and Canada, and *P. stimpsoni* is among one of the many *Panulirus* species heavily harvested in temperate and tropical oceans throughout the world (Cobb and Wang, 1985). Using an expression clone of the lobster tropomyosin, we demonstrated that sera from sub-

Table 1. Amino acid composition of tropomyosins from various shellfish, fruitfly, and chicken.*

Amino acid	<i>Pan s I</i> [†]	<i>Hom a I</i> [‡]	<i>Met e I</i> [§]	Ha sTm [‡]	Fruitfly	Chicken
Alanine	31	33	31	33	25	34
Cysteine	0	0	1	0	3	1
Aspartic acid	17	18	17	16	22	25
Glutamic acid	55	55	55	57	51	54
Phenylalanine	4	3	4	3	5	1
Glycine	4	4	5	4	3	3
Histidine	1	1	1	1	1	1
Isoleucine	4	6	4	8	7	9
Lysine	25	28	25	29	35	40
Leucine	33	33	33	31	28	38
Methionine	6	8	6	8	8	4
Asparagine	16	16	18	15	13	6
Proline	0	0	0	0	0	0
Glutamine	16	17	17	17	19	12
Arginine	21	21	22	21	17	13
Serine	14	15	15	13	14	17
Threonine	10	9	9	11	14	10
Valine	13	13	14	13	17	9
Tryptophan	0	0	0	0	0	0
Tyrosine	4	4	4	4	2	7
Total	274	284	281	284	284	284

* Determined from deduced amino acid sequences from cDNA clones.
† This study.
‡ *Homarus americanus* slow muscle tropomyosin (Mykles et al., 1997).
§ Leung et al., 1994.
|| Amino acid sequences of fruitfly tropomyosin I (Basi and Storti, 1986) and chicken fast muscle tropomyosin (Goodling et al., 1987) are used for comparison.

jects with a clinical history of crustacean allergy have a high IgE reactivity against the lobster tropomyosin. The IgE antibodies from the crustacean-sensitive subjects show reactivity to a 34-kDa native lobster muscle protein as well as the recombinant lobster tropomyosin *Pan s I* and *Hom a I*. Moreover, data from inhibition experiments also show that sera absorbed with recombinant lobster tropomyosin or shrimp tropomyosin lost their IgE reactivities against lobster muscle, suggesting that the epitopes between lobster and shrimp tropomyosin are highly similar, if not identical.

The results from this study demonstrate a muscle protein tropomyosin as a lobster allergen, as well as the possibility of tropomyosin as a major common seafood allergen. To our knowledge, this is the first report to demonstrate the IgE cross-reactivity at the molecular level between two popular crustacean seafood, namely lobster and shrimp, and also identify the cross-reactive molecule as a highly conserved muscle protein tropomyosin.

Tropomyosins are a group of proteins with multiple isoforms found in both muscle and nonmuscle

cells. They show tissue-specific functions both in vertebrates (Smillie, 1979) and in crustaceans (Miyazaki et al., 1992). In muscle, tropomyosin interacts with actin and troponin T to regulate the calcium-dependent system of muscle contraction (White et al., 1987). Tropomyosins are elongated proteins composed of a double-stranded α -helical coiled structure, which binds themselves in a head-to-tail manner. Although tropomyosins are highly conserved regulatory proteins, the functional domains of the proteins are different. For instance, the actin and troponin-binding sites and sequences in head-to-tail polymerization are divergent regions in the proteins (White et al., 1987). Unlike these divergent regions, the allergenic epitopes were found to be highly conserved in the shrimp and lobster tropomyosins in the present study. These allergenic epitopes may also be conserved in tropomyosins in our common edible shellfish.

Comparison of amino acid sequences of *Pan s I* and *Hom a I* with the shrimp tropomyosin *Met e I* indicates that these proteins are very similar, with 98.2% identity between *Pan s I* and *Met e I*, 94.7%

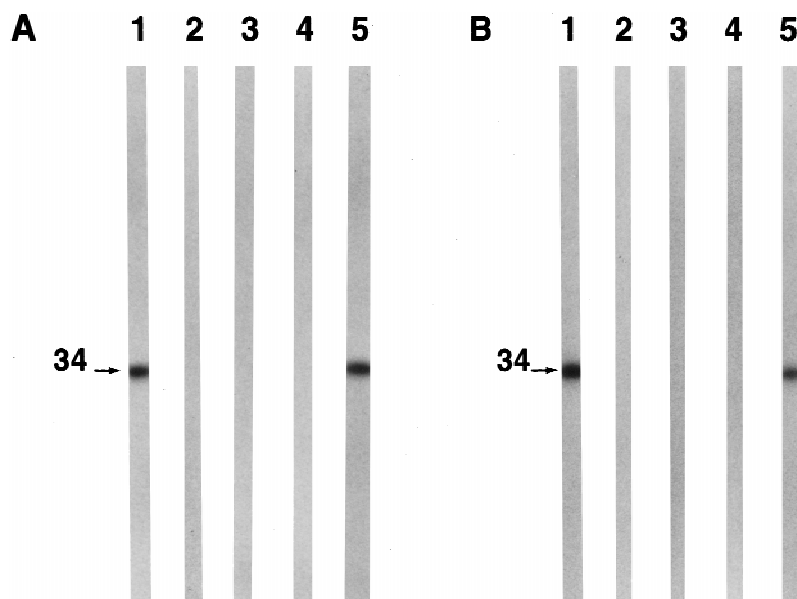


Figure 3. Specific inhibition of IgE reactivity against (A) *P. homarus* and (B) *H. americanus* proteins by *Pan s I*, *Hom a I*, and *Met e I*. Note the presence of a reactive band with unabsorbed sera at 34 kDa (lane 1) and the loss of reactivity when the sera were absorbed by recombinant proteins of *Pan s I* (lane 2), *Hom a I* (lane 3), and *Met e I* (lane 4). Sera absorbed by an irrelevant control recombinant protein, PDC-E2, did not inhibit the reactivity (lane 5).

identity between *Hom a I* and *Met e I*, and 97.5% identity between *Pan s I* and *Hom a I*. The degree of identity is much lower when compared with tropomyosin of fruitfly and chicken (Basi and Storti, 1986, Goodling et al., 1987) (Table 2). Thus, the significant amino acid sequence identity of these shellfish proteins can be accounted for by their close phylogenetic relationship as well as the physiologic, biochemical, and functional similarities of these muscle proteins. Therefore, it is not surprising that patients allergic to shellfish tropomyosins have IgE reactivities to *Pan s I*, *Hom a I*, as well as *Met e I*. Moreover, the high amino sequence identity of *Pan s I* and *Met e I* with *Hom a I* (Mykles et al., 1997), the fast muscle tropomyosin of *H. americanus*, suggests that *Pan s I* and *Met e I* correspond to the fast muscle tropomyosin of *P. stimpsoni* and *M. ensis*, respectively.

Experimental Procedures

Sera

Sera from 10 patients with crustacean allergies were obtained from the sera bank at the Division of Rheumatology/Allergy and Clinical Immunology at the University of California, Davis. These sera were shown to have IgE reactivity to several seafood including shrimp, lobsters, and crabs (Leung et al., 1996). In addition, sera from five normal volunteers without any previous history of seafood allergy were used as controls.

Construction of cDNA library

Total RNA was isolated from the abdominal muscle of *P. stimpsoni* by the guanidium isothiocyanate method using the TRIzol reagent (Life Technologies, Gaithersburg, Md.). The procedures recommended by the manufacturer were followed by using 0.5 g of muscle tissue in 5 ml of reagent. The cDNA library was then constructed using the CapFinder PCR cDNA Library Construction Kit (Clontech, Palo Alto, Calif.) according to manufacturer's instruction. After being ligated into *EcoRI* ends of λ gt11, the library was packaged in λ phage using the Gigapack III Gold Packaging Extract (Stratagene, La Jolla, Calif.).

Screening of *Panulirus stimpsoni* cDNA library

Using sera from patients with crustacean allergies as probes, the *P. stimpsoni* cDNA library was plated on LB agar by infecting *E. coli* Y1090 with λ gt11 at a density of 20,000 to 30,000 per plate. Plaques were induced for 8 h on nitrocellulose filters that had been previously saturated with 1 mM isopropyl β -galactopyranoside (IPTG) (Alexus, San Diego, Calif.). Thereafter the filters were blocked with blotto (3% milk in phosphate-buffered saline, PBS) and incubated with sera from patients with crustacean allergy (1:10 dilution) at 4°C for 8–10 h. The filters were then washed with PBS containing 0.05% Tween-20 three times and then incubated with ^{125} I-labeled antihuman IgE (Sanofi-Pasteur Diagnostics, Ohaska, Minn.) at 4°C for 8–10 h. The

Table 2. Amino acid identity of shellfish, fruitfly, and chicken tropomyosin.

% Identity*	<i>Pan s I</i> [†]	<i>Hom a I</i> [‡]	<i>Met e I</i> [§]	Ha sTm [†]	Fruitfly	Chicken
<i>Pan s I</i>	100					
<i>Hom a I</i>	97.5	100				
<i>Met e I</i>	98.2	94.7	100			
Ha sTm	92.3	94.7	89.3	100		
Fruitfly	66.8	67.9	65.5	68.2	100	
Chicken	47.6	48.2	46.4	47.9	39.7	100

* Percentage of identity of the peptide sequence expressed as the nearest decimal place.
† This study.
‡ *Homarus americanus* slow muscle tropomyosin (Mykles et al., 1997).
§ Leung et al., 1994.
|| Amino acid sequences of fruitfly tropomyosin I (Basi and Storti, 1986) and chicken fast muscle tropomyosin (Goodling et al., 1987) are used for comparison.

filters were washed as before and exposed to x-ray film. Positive signals were purified to homogeneity. A particular clone, designated as *Pan s I*, was chosen for further characterization.

Nucleotide sequence determination of Pan s I

Phase DNA was isolated from the purified IgE-reactive λgt11 clone as described in Maniatis et al. (1982), and the cDNA insert was excised by restriction enzyme *EcoRI*. The cDNA was then gel-purified on low melting temperature agarose and ligated into the *EcoRI* site of plasmid vector pSK- (Stratagene). After transformation, miniprep DNA was prepared from transformants to test for the correct size of insert using a QIAprep spin plasmid miniprep kit (Qiagen, Chatsworth, Calif.). The nucleotide sequence of the cDNA was determined by cycle sequencing of the plasmid DNA with *AmpliTaq* DNA polymerase and dye-labeled terminators using the ABI PRISM Dye terminator cycle sequencing ready reaction kit (Perkin-Elmer) and sequencing primers T3 and T7 as well as derived primers TM3 (5'-GAATTCATGAAGCTG-GAGAAGGA-3') and TM4 (5'-GAATTCGTAGC-CAGACAGTTCGA-3') and L1 (5'-ACCTCGTCG-TATTCCTGT-3') derived from *M. ensis* tropomyosin (GenBank database, accession number U08008). Nucleotide sequence were analyzed with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems Inc., Foster City, Calif.). The nucleotide sequence was further edited by the software Sequence Editor, version 1.0.3 (Applied Biosystems Inc.) (Horton et al., 1994; Daniel, 1996).

Expression of cDNA

We cloned cDNA encoding the IgE-reactive clone *Pan s I* into the *EcoRI* site of plasmid expression

vectors pGEX (Smith and Johnson, 1988). Transformants were selected and colony immunoassay was performed using sera from patients with shrimp allergy. Briefly, transformants were picked onto nitrocellulose filters, and the filters were incubated on LB agar plates containing 25 µg/ml ampicillin for 10 h at 37°C and then induced by transferring the filter to LB agar plates containing 25 µg/ml ampicillin plus 1 mM IPTG. After 4 h of incubation at 37°C, cells were lysed by chloroform vapor and the filters were incubated with blotto for blocking. After blocking, the filters were incubated with sera from patients with crustacean allergy (1:10 dilution) at 4°C for 8–10 h. The filters were then washed with PBS containing 0.05% Tween-20 three times and then incubated with ¹²⁵I-labeled antihuman IgE (Sanofi-Pasteur Diagnostics) at 4°C for 8–10 h. The filters were washed as before and exposed to x-ray film. Colonies corresponding to positive signals on the x-ray film were chosen for immunoblotting.

Determination of molecular weight of Pan s I by immunoblotting

Positive expression clones were grown in LB containing 25 µg/ml ampicillin at 37°C overnight and then induced by 1 mM IPTG for 4 h at 37°C. The cell lysate was resolved on 8% SDS-PAGE, which was then transferred onto nitrocellulose filter as described by Leung et al. (1994, 1996). Sera from patients with crustacean allergy (1:10 dilution) were then used to probe the filters, and IgE reactivity was determined using ¹²⁵I-labeled antihuman IgE (Sanofi-Pasteur Diagnostics, Inc.). After washing and exposing the x-ray film, the position of the positive band was located and its molecular weight

was determined with reference to the molecular weight marker.

Purification of Pan s I recombinant protein

A 500-ml overnight culture of a pGEX-expressing clone of the IgE-reactive *Pan s I* protein was induced with 1 mM IPTG, and the cells were harvested. The cells were then suspended in 5 ml of lysis buffer (1% Triton X-100, 1% Tween-20, 10 mM dithiothreitol) and sonicated. The cell lysate was centrifuged at 6000 *g* for 15 min to pellet the debris, and the supernatant was added to glutathione agarose (Sigma, St. Louis, Mo.). After incubating for 2 h on a rocker, the glutathione agarose was washed three times with PBST (PBS containing 1% Triton X-100, pH 7.3), and the bound protein was eluted using 2 ml of 5 mM reduced glutathione (Sigma) in 50 mM Tris-HCl, pH 8.0. The amount of purified protein was measured using the protein assay reagent (Bio-Rad, Richmond, Calif.).

Allergen extracts

Live specimens of *P. homarus* and *H. americanus* were obtained from seafood markets in Hong Kong and Davis, California, respectively. The abdominal muscle was removed, homogenized in PBS, pH 7.2, with a polytron, and mixed for 8 h at 4°C. Thereafter, the lysate was centrifuged at 5000 *g* for 15 min, and the supernatant was stored in small aliquot at -20°C until use. The crude homogenate was assayed for total protein using a protein assay kit (Bio-Rad).

cDNA clones

The cDNA clone encoding the major heat-stable shrimp allergen *Met e I* from *M. ensis* (Leung et al., 1994) has been described. A cDNA clone encoding the *H. americanus* tropomyosin was used to obtain an expression clone of the lobster fast muscle tropomyosin isoform (Cotton and Mykles, 1993; Mykles et al., 1997). Briefly, PCR (Saiki et al., 1988) was performed to amplify the lobster tropomyosin coding sequence using primer pairs 5'-GGAATTCATGGACGCAATCAAGAAGAAG-3' and 5'-GGAATTCGTAGCCAGACAGTTCGCT-3' as forward and reverse primers, respectively. These primers were derived from sequence of the *H. americanus* fast muscle tropomyosin (Mykles et al., 1997). Forward and reverse primers were designed with *EcoRI* restriction sites at their ends. PCR was performed for 30 cycles at 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min with 10 ng of template

DNA, 1 µM of each primer, 200 µM of dNTPs, 5 units of *Taq* polymerase in PCR buffer containing 2 mM MgCl₂. PCR-amplified DNA was cloned into the *EcoRI* site of frame-shifted plasmid vector pGEX (Pharmacia) and transformed into *E. coli*. *E. coli* expressing the lobster tropomyosin was detected by colony immunoassay with shrimp allergy sera using procedures described previously (Leung et al., 1994). Colonies showing positive reactivity were purified to homogeneity.

Analysis of IgE reactivity against lobster extract

Muscle homogenate of *H. americanus* (200 µg per gel) was resolved by SDS-PAGE using a 10% separating gel and 4% stacking gel. Resolved proteins were transferred onto nitrocellulose filters as described by Leung et al. (1995). The nitrocellulose filter was then cut into 3-mm strips and incubated with sera from subjects with crustacean allergy and normal sera as controls at 4°C overnight. The nitrocellulose strips were then washed with PBS containing 0.05% Tween-20 (PBS-Tween) three times, for 10 min each. IgE reactivity was detected using ¹²⁵I-labeled antihuman IgE (Sanofi-Pasteur Diagnostics, Inc.).

Specific inhibition of IgE reactivity by recombinant lobster tropomyosin

Sera (at 1:10 dilution in PBS containing 3% milk powder) from subjects with crustacean allergy were incubated for 8–12 h at 4°C with 100 µg of recombinant lobster tropomyosin *Pan s I* and *Hom a I*, and recombinant shrimp tropomyosin *Met e I* in a final volume of 1 ml. Sera were also incubated with 100 µg of recombinant glutathione S-transferase as controls. The absorbed sera were then tested for their IgE reactivities against lobster extract and recombinant lobster tropomyosin by immunoblotting.

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