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# Molecular Cloning of Tropomyosins Identified as Allergens in Six Species of Crustaceans

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Although tropomyosin is known to be a major allergen of crustaceans, its structural information is limited to only five species. In this study, tropomyosin was confirmed to be a major allergen in six species of crustaceans (black tiger prawn, kuruma prawn, pink shrimp, king crab, snow crab, and horsehair crab) by immunoblotting. Then, the amino acid sequences of tropomyosins from these crustaceans were elucidated by a cDNA cloning technique. Sequence data for crustacean tropomyosins including the obtained results reveal that fast tropomyosins are contained in shrimps (or prawns) and lobsters, slow tropomyosins in crabs, and both tropomyosins in crayfishes and hermit crabs. Although fast and slow tropomyosins share a high sequence identity (about 90%) with each other, significant differences are observed in specific regions between both tropomyosins.

**KEYWORDS: Allergen; cDNA cloning; crab; cross-reactivity; crustacean; prawn; shrimp; tropomyosin**

## INTRODUCTION

Crustaceans are widely consumed as delicious foods throughout the world. However, they are simultaneously recognized as one of the most common causes of immunoglobulin E (IgE)-mediated food allergy. Following their ingestion, hypersensitive reactions, such as urticaria, asthma, diarrhea, and anaphylaxis, are immediately induced in allergic individuals. Most edible crustaceans are members of the order Decapoda composed of two suborders, Dendrobranchiata (including shrimps or prawns) and Pleocyemata (including shrimps or prawns, crayfishes, lobsters, hermit crabs, and crabs), and hence previous studies on crustacean allergens have been performed with decapods. So far, the major allergen of crustaceans has been identified at the molecular level as tropomyosin, a 35−38 kDa protein constituting thin myofilaments together with actin and troponin, in the following six species: Indian white shrimp, *Penaeus indicus* (*1*); brown shrimp, *Peanaeus aztecus* (*2*); sand shrimp, *Metapenaeus ensis* (*3*); American lobster, *Homarus americanus* (*4*); spiny lobster, *Panulinus stimpsoni* (*4*); and red crab, *Charybdis feriatus* (*5*). The three species of shrimps belong to the suborder Dendrobranchiata and the rest to the suborder Pleocyemata. Although tropomyosin is assumed to be a major allergen in common with decapod crustaceans, no experimental data are available on allergens in shrimps belonging to the suborder Pleocyemata and hermit crabs including commercially important species such as king crab.

Crustacean tropomyosins show IgE cross-reactivity with one another and also with those from various invertebrates including mollusks, house dust mites, and cockroaches (*6*−*12*). Obviously, the first step toward molecular understanding of the cross-reactivity among tropomyosins from various sources is the elucidation of their amino acid sequences. As for crustacean tropomyosins, those from brown shrimp (*13*), sand shrimp (*3*), American lobster (*14*, *15*), spiny lobster (*4*), and red crab (*5*) have already been clarified for their amino acid sequences. The tropomyosins from two species of shrimps, American lobster and spiny lobster, share an extremely high sequence identity (>98%) with one another. In accordance with this, the eight IgE-binding epitopes proposed for brown shrimp tropomyosin (Pen a 1) (*10*, *16*) are completely conserved in the tropomyosins from the other three species, except that the spiny lobster tropomyosin has one alteration in one epitope. On the other hand, the red crab tropomyosin bears a somewhat different amino acid sequence, and its sequence identity with those of the above four species is about 90%. Importantly, the red crab tropomyosin has as many as six alterations in the region 44−55 corresponding to one of the Pen a 1 epitopes. At present, however, it is unknown whether the amino acid sequence features of the red crab tropomyosin are common to crab tropomyosins.

In view of the circumstances described above, this study was initiated to obtain further evidence that tropomyosin is a major allergen in decapod crustaceans using the following six species widely consumed in Japan: two species of the suborder Dendrobranchiata, black tiger prawn (*Penaeus monodon*) and kuruma prawn (*Penaeus japonicus*), and four species of the suborder Pleocyemata, pink shrimp (*Pandalus eous*), king crab (*Paralithodes camtschaticus*) (a kind of hermit crabs), snow crab (*Chionoecetes opilio*), and horsehair crab (*Erimacrus isenbekii*).

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**Table 1.** Designations and Nucleotide Sequences of the Primers Used for Molecular Cloning of Crustacean Tropomyosins

| designation of primer | nucleotide sequence of primer | corresponding amino acid sequence[a] |
|---|---|---|
| cru-f1 | 5′-ATCAAGAAGAAGATGCAGGCG-3′ | 4-IKKKMQA-10 |
| cru-f2 | 5′-AGAAGGCCAACCAGCGCGAGGA-3′ | 212-EKANQREE-219 |
| kuruma-f | 5′-AGAAGGCTAACCAGCGCGAGGA-3′ | 212-EKANQREE-219 |
| cru-r1 | 5′-TCTTCGAGCCTGTCGACCTC-3′ | 251-KEVDRLED-258 |
| cru-r2 | 5′-GGATGTTAGCCTTCAGCAAGGATT-3′ | 62-ESLLKANIQ-70 |
| pink-s | 5′-CGTTAGAGAGAGCCTTGTCC-3′ | 74-KDKALSNA-81 |
| pink-r | 5′-GGTGTTGGTTAGCCTTCAGC-3′ | 64-LLKANTQL-71 |
| snow-f | 5′-GGAACAGATCAAGACCCTTGCC-3′ | 222-KEQIKTLA-229 |
| snow-r | 5′-TATGGGTAGCGGCGGACAACTGCT-3′ | 62-ESLLKANIQ-70 |
| horsehair-s | 5′-CCTCCTCGAGAAGCTGGATG-3′ | 91-RIQLLEED-98 |
| horsehair-r | 5′-CACCTCACCCTCGGCATTCT-3′ | 79-SNAEGEV-85 |
| king-f1 | 5′-CGAGGAGGAGGTTCACGGCCTT-3′ | 39-AEEEVHGL-46 |
| king-f2 | 5′-TGAGGAGGAGATTCGCCTTACC-3′ | 39-IEEEIRLT-46 |
| king-r1 | 5′-TTGCGTATTCGCCTTAAGCAGGGA-3′ | 63-SLLKANTQ-70 |
| king-r2 | 5′-CTTAGTGTTAGCCAGAGATAGCTG-3′ | 63-QLSLANTK-70 |
| AP1 | 5′-CCATCCTAATACGACTCACTATAGGGC-3′ | |
| AUAP | 5′-GGCCACGCGTCGACTAGTAC-3′ | |
| AAP | 5′-GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG-3′ | |

[a] Refer to **Figure 2** for amino acid sequences.

American lobster, for which the major allergen has previously been established to be tropomyosin (*4*), was used as a reference. Subsequently, amino acid sequences of tropomyosins from the six species of crustaceans were elucidated by a cDNA cloning technique. We report here the identification of tropomyosins as allergens in six species of decapod crustaceans and the primary structural features of their tropomyosins in comparison with the known crustacean tropomyosins.

## MATERIALS AND METHODS

**Crustaceans.** Live specimens of kuruma prawn, pink shrimp, American lobster, and horsehair crab were purchased at the Tokyo Central Wholesale Market. Live specimens of black tiger prawn imported from Vietnam and those of king crab and snow crab caught along the coasts of Hokkaido, Japan, were kindly supplied from Nippon Suisan Kaisha. Abdominal muscle was obtained from each live specimen of black tiger prawn, kuruma prawn, pink shrimp, and American lobster and both leg muscle and chest protection muscle from king crab, snow crab, and horsehair crab. Muscle samples for extraction were stored at −20 °C until used, and those for molecular cloning experiments were immediately frozen in liquid nitrogen and stored at −80 °C until used.

**Preparation of Heated Extracts.** Each muscle sample was homogenized with 4 volumes of 0.6 M KCl in 0.01 M phosphate buffer (pH 7.0). Tropomyosin, a target protein in this study, is heat-stable. Therefore, the homogenate was then heated in a boiling water bath for 10 min and centrifuged at 18000*g* for 20 min to remove insoluble proteins. The supernatant thus obtained was used as heated extract. Protein concentrations of the heated extracts were estimated according to the method of Lowry et al. (*17*), using bovine serum albumin as a standard.

**Purification of American Lobster Tropomyosin.** An acetone powder of myofibrillar proteins was prepared from the abdominal muscle of American lobster as reported by Greaser and Gergely (*18*) and extracted with 25 mM Tris-HCl buffer (pH 8.0) containing 1 M KCl, 0.1 mM $CaCl_2$, and 1 mM dithiothreitol. The extract was subjected successively to salting-out with ammonium sulfate (50−60% saturation) and isoelectrical precipitation (pH 4.6) essentially according to the method of Cummins and Perry (*19*). Finally, small amounts of impurities were removed by reverse-phase HPLC on a TSKgel ODS-120T column (0.46 × 25 cm; Tosoh, Tokyo, Japan), which was eluted at a flow rate of 1 mL/min with a linear gradient of acetonitrile (38.5−49.0% in 50 min) in 0.1% trifluoroacetic acid. The homogeneity of the final preparation was supported by SDS-PAGE. American lobster has previously been reported to contain three types of tropomyosin (fast, slow-twitch and slow-tonic) (*14*, *15*). Because the abdominal muscle of American lobster is mostly composed of fast muscle fibers (*14*), our purified preparation was judged to be fast-type tropomyosin.

**Human Sera.** Sera were donated from 10 crustacean-allergic patients. The patients were all diagnosed to be allergic to crustaceans on the basis of the determined capsulated hydrophilic carrier polymer−radioallergosorbent test (CAP-RAST) classes of 2−5 against shrimp or both shrimp and crab as well as on the experiences of immediate hypersensitive reactions after ingestion of crustaceans. Written informed consent was obtained from each patient. In this study, sera from five healthy volunteers without adverse reactions after ingestion of any foods were pooled and used as a control. All sera were stored at −20 °C until used.

**Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE).** SDS-PAGE was performed on a PhastSystem apparatus (GE-Healthcare Bio-Sciences, Piscataway, NJ) according to the manufacturer's instructions. Ready-made gels (PhastGel Gradient 8-25) and ready-made buffer strips (PhastGel SDS Buffer Strips) were purchased from GE-Healthcare Bio-Sciences. Prior to electrophoresis, each sample was dissolved in 0.0625 M phosphate buffer (pH 7.5) containing 2.5% SDS and 5% dithiothreitol and heated at 100 °C for 10 min. Precision Plus Protein Standards (Bio-Rad Laboratories, Hercules, CA) were run as a reference, along with samples. After running, the gel was stained with Coomassie Brilliant Blue R-250.

**Immunoblotting and Inhibition Immunoblotting.** Immunoblotting was performed as described in our previous paper (*20*). Briefly, the proteins separated by SDS-PAGE were electrotransferred from the gel to a nitrocellulose membrane, which was reacted successively with primary and secondary antibodies. To detect tropomyosin, an antiserum (diluted 1:40000) raised in rabbits against king crab tropomyosin, which was a kind gift from Dr. H. Ushio of our university, was used as a primary antibody and horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (diluted 1:20000; Kirkegaard and Perry Laboratories, Gaithersburg, MD) as a secondary antibody. On the other hand, patient or control serum (diluted 1:500) and horseradish peroxidase-conjugated goat anti-human IgE antibody (diluted 1:10000; Kirkegaard and Perry Laboratories) were used as primary and secondary antibodies, respectively, to detect IgE-binding proteins. Blots were visualized using an ECL Plus Western Blotting Detection System (GE-Healthcare Bio-Sciences) and an ECL Mini Camera (GE-Healthcare Bio-Sciences), as recommended by the manufacturer. For inhibition IgE-immunoblotting, patient serum (diluted 1:250) was preincubated with an equal volume of American lobster tropomyosin solution (20 $\mu$g/mL) at 37 °C for 1 h and used as a primary antibody.

**Primer Design, Polymerase Chain Reaction (PCR), and Nucleotide Sequencing.** Designations and nucleotide sequences of the primers used in this study are shown in **Table 1**. Both forward (cru-f1) and reverse (cru-r1) primers were designed from the known nucleotide sequences of the crustacean tropomyosin cDNAs. The nucleotide

sequence of cru-f1 is completely identical with the cDNAs encoding tropomyosins from brown shrimp (accession number of the DDBJ/EMBL/GenBank nucleotide databases: DQ151457) and red crab (AF061783) and three types of tropomyosin from American lobster (AF034954 for fast tropomyosin, AF034953 for slow-twitch tropomyosin, and AY521627 for slow-tonic tropomyosin). On the other hand, the nucleotide sequence of cru-r1 is identical with the cDNAs encoding tropomyosins from sand shrimp (U08008) and American lobster and spiny lobster (AF030063) but is different by one nucleotide from the cDNAs encoding tropomyosins from brown shrimp and red crab. Except for three primers, AP1 adapter primer (AP1), abridged universal anchor primer (AUAP), and abridged anchor primer (AAP), the other primers were designed on the basis of the determined partial nucleotide sequences. PCR amplifications were all performed using HotMaster Taq DNA polymerase (Eppendorf, Hamburg, Germany) under the following conditions: 94 °C for 2 min; 35 cycles of 94 °C for 20 s, 55 °C for 20 s and 72 °C for 1 min; and 72 °C for 7 min. The PCR products were subcloned into the pT7Blue-2 T-vector (Novagen, Darmstadt, Germany), and at least three clones were analyzed for nucleotide sequences using a PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) and a PRISM 310 genetic analyzer (Applied Biosystems).

**Cloning Experiments for Tropomyosins of Black Tiger Prawn, Kuruma Prawn, King Crab, and Snow Crab.** Total RNA was extracted from 2 g of each muscle sample with the TRIzol reagent (Invitrogen, Carlsbad, CA), and poly(A)$^+$ mRNA was purified by affinity chromatography on oligo(dT)-cellulose using an mRNA Purification Kit (GE-Healthcare Bio-Sciences). A small aliquot (1 $\mu$g) of the purified mRNA was converted to double-strand cDNA, followed by ligation of an AP1 adapter at both 3′ and 5′ ends, using a Marathon cDNA Amplification Kit (Clontech Laboratories, Palo Alto, CA). The Marathon cDNA library thus constructed was subjected to PCR as a template. At first, intermediate cDNA fragments were amplified using the forward primer (cru-f1) and the reverse primer (cru-r1). All amplified products were subcloned into the pT7Blue-2 T-vector and sequenced as described above. Then, the remaining 3′ region was amplified using the gene-specific forward primer (cru-f2 for black tiger prawn and snow crab, kuruma-f for kuruma prawn, king-f1 for king crab fast-type isoform or king-f2 for king crab slow-tonic-type isoform) and the AP1 primer and the remaining 5′ region using the gene-specific reverse primer (cru-r2 for black tiger prawn and kuruma prawn, king-r1 for king crab fast-type isoform, king-r2 for king crab slow-tonic-type isoform or snow-r for snow crab) and the AP1 primer. In the case of snow crab, nested PCR was additionally performed to amplify the 3′ remaining region using the gene-specific forward primer (snow-f) and the AP1 primer. All amplified products were subjected to subcloning and sequencing.

**Cloning Experiments for Tropomyosins of Pink Shrimp and Horsehair Crab.** Molecular cloning of pink shrimp and horsehair crab tropomyosins was simply performed by the rapid amplification of cDNA ends (RACE) method using total RNA extracted from each muscle sample, without construction of the Marathon cDNA library. For 3′RACE, first-strand cDNA was synthesized from 5 $\mu$g of total RNA using the 3′RACE System for Rapid Amplification of cDNA Ends (Invitrogen) as described in the manufacturer's manual and used as a template. Amplification was carried out using the cru-f1 primer and the AUAP primer. 5′RACE was performed using the 5′RACE System for Rapid Amplification of cDNA Ends (Invitrogen) according to the manufacturer's instructions. First-strand cDNA was synthesized from 5 $\mu$g of total RNA using the gene-specific reverse primer (pink-s for pink shrimp or horsehair-s for horsehair crab). The gene-specific reverse primer (pink-r for pink shrimp or horsehair-r for horsehair crab) and the AAP primer were subjected to 5′RACE reaction using the synthesized first-strand cDNA as a template. All amplified products were subcloned and sequenced.

## RESULTS AND DISCUSSION

**Identification of Tropomyosins as Allergens in Crustaceans.** As analyzed by SDS-PAGE, a prominent protein of 37 kDa, which is comparable in molecular mass to American lobster tropomyosin used as a reference, was detected in all of the heated extracts, except for that from the chest protection muscle of king crab (**Figure 1A**). Irrespective of the species and the muscle types, the 37 kDa protein reacted with the antiserum against king crab tropomyosin (**Figure 1B**), allowing us to conclude that the 37 kDa protein is tropomyosin of each crustacean muscle. In the case of the chest protection muscle of king crab, two proteins of about 37 kDa with a considerably weaker staining intensity than tropomyosins in the other samples were observed in SDS-PAGE (**Figure 1A**). The two proteins also reacted with the antiserum against king crab tropomyosin (**Figure 1A**). Importantly, two different tropomyosins (fast and slow-tonic tropomyosins) exist only in the chest protection muscle of king crab, as described in detail below. Taken together, it is reasonable to consider that the two proteins of about 37 kDa observed in the chest protection muscle of king crab are tropomyosin isoforms. IgE-immunoblotting, using the pooled serum from 10 crustacean-allergic patients and 4 individual patient sera, showed that all tropomyosins, except for the two tropomyosin isoforms in the chest protection muscle of king crab, are IgE-reactive, regardless of the patient sera (**Figure 1C**). When the pooled control serum was used, no blots were detected in any of the heated extracts (data not shown). As for the two isoforms in the chest protection muscle of king crab, their IgE reactivity was established by the results with the pooled patient serum and the patient 2 and 4 sera. However, only one blot was observed when patient 1 and 3 sera were used, suggesting that only either one of the two isoforms is reactive with IgE in the patient 1 and 3 sera or that the two blots have appeared as one blot due to poor resolution. Apart from this problem to be clarified in future, it is important to note that tropomyosin seemed to be almost the sole IgE-reactive protein in all of the crustaceans examined, although another IgE-reactive protein of about 30 kDa was also detected in the chest muscle of king crab only when the patient 2 serum was used. In inhibition immunoblotting experiments using American lobster tropomyosin as an inhibitor, the patient sera completely lost their reactivity to tropomyosins from six species of crustaceans (only the results with the pooled patient serum are shown in **Figure 1D**), demonstrating the cross-reactivity between tropomyosins from American lobster and the other crustaceans.

Our results may be as expected from the current opinion that tropomyosin is a major and cross-reactive allergen in crustaceans (*7*, *11*, *12*). However, it should be noted that, of the six species of crustaceans used in this study, pink shrimp belonging to the suborder Pleocyemata and king crab (a kind of hermit crabs) are taxonomically distinct from the crustaceans so far studied on allergens. Therefore, this study is of value in providing experimental evidence that the current opinion is true for almost all crustaceans belonging to the order Decapoda.

**Nucleotide Sequences of Crustacean Tropomyosin cDNAs.** The cDNAs encoding tropomyosins from black tiger prawn, kuruma prawn, king crab, and snow crab were successfully cloned by PCR using the Marathon cDNA library as a template and those encoding tropomyosins from pink shrimp and horsehair crab by both 3′RACE and 5′RACE using total RNA. In the case of the chest protection muscle of king crab, two different cDNAs (encoding fast and slow-tonic tropomyosins as described below in more detail) were obtained; three of the five clones analyzed corresponded to the fast tropomyosin and the rest to the slow-tonic tropomyosin. On the other hand, only one kind of cDNA was cloned from the other samples. The cDNA from the leg muscle of king crab was identical with one

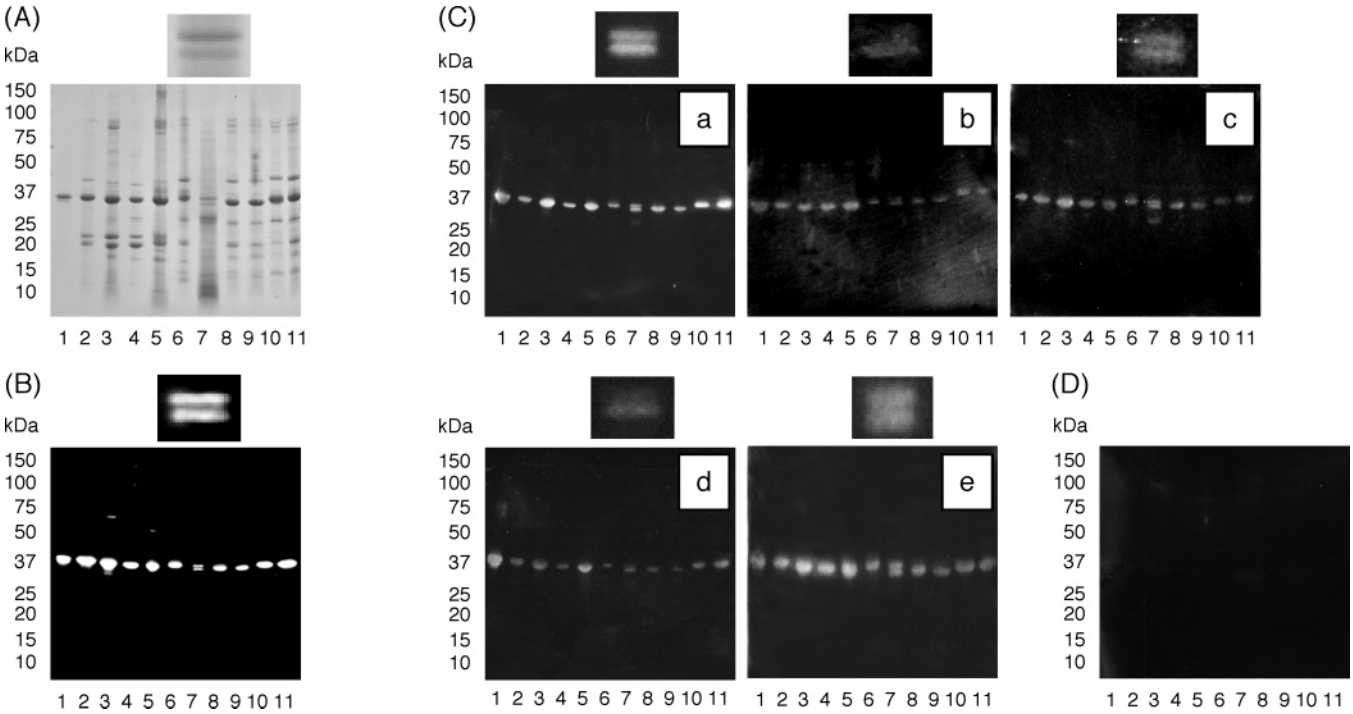


**Figure 1.** Analysis of heated extracts from various crustaceans by SDS-PAGE (**A**), IgG-immunoblotting (**B**), IgE-immunoblotting (**C**), and inhibition immunoblotting (**D**). Lanes: 1, tropomyosin purified from American lobster; 2, extract from American lobster; 3, extract from black tiger prawn; 4, extract from kuruma prawn; 5, extract from pink shrimp; 6, extract from king crab leg muscle; 7, extract from king crab chest protection muscle; 8, extract from snow crab leg muscle; 9, extract from snow crab chest protection muscle; 10, extract from horsehair crab leg muscle; 11, extract from horsehair crab chest protection muscle. A magnification of the 37 kDa region in lane 7 (extract from king crab chest protection muscle) is shown above each figure in **A**, **B**, and **C**. (**A**) Heated extracts (0.3 $\mu$L each) with the following protein concentrations were subjected to SDS-PAGE: 3.6 mg/mL for American lobster, 6.6 mg/mL for black tiger prawn, 3.0 mg/mL for kuruma prawn, 9.8 mg/mL for pink shrimp, 3.8 mg/mL for king crab leg muscle, 7.8 mg/mL for king crab chest protection muscle, 4.2 mg/mL for snow crab leg muscle, 3.2 mg/mL for snow crab chest protection muscle, 3.2 mg/mL for horsehair crab leg muscle, and 5.6 mg/mL for horsehair crab chest protection muscle. (**B**) Antiserum against king crab tropomyosin was used as a primary antibody. (**C**) Patient sera were used as a primary antibody: **a**, pooled serum from 10 patients; **b**, patient 1; **c**, patient 2; **d**, patient 3; **e**, patient 4. (**D**) Pooled serum (diluted 1:250) from 10 patients was preincubated with an equal volume of inhibitor (American lobster tropomyosin) solution (20 $\mu$g/mL) and used as a primary antibody.

(encoding the fast tropomyosin) of the two cDNAs from the chest protection muscle. The same cDNA was found in the leg and chest protection muscles of snow crab, whereas the cDNAs from two types of muscles of horsehair crab differed from each other. These results agreed well with the data obtained in SDS-PAGE and immunoblotting showing the presence of two tropomyosin isoforms only in the chest protection muscle of king crab (**Figure 1**).

Although not shown in this paper to save space, the determined nucleotide sequences of the full-length cDNAs have been deposited in the DDBJ/EMBL/GenBank databases under the following accession numbers AB270629 for black tiger prawn tropomyosin (1260 bp), AB270630 for kuruma prawn tropomyosin (1299 bp), AB270631 for pink shrimp tropomyosin (1050 bp), AB270632 for king crab fast tropomyosin (1493 bp), AB270633 for king crab slow-tonic tropomyosin (1559 bp), AB270634 for snow crab tropomyosin (1148 bp), AB270635 for horsehair crab slow-twitch tropomyosin (1137 bp), and AB270636 for horsehair crab slow-tonic tropomyosin (1016 bp). Irrespective of the cDNAs, an open reading frame contains 852 bp coding for 284 amino acid residues.

**Amino Acid Sequences of Crustacean Tropomyosins.** The amino acid sequences of eight tropomyosins from six species of crustaceans were elucidated by a cDNA cloning technique. This is the first report of the amino acid sequences of tropomyosins from shrimp (pink shrimp) belonging to the suborder Pleocyemata and hermit crab (king crab), making it possible to understand the overall features of the amino acid sequences of tropomyosins from edible crustaceans (decapod crustaceans). The known amino acid sequences of crustacean tropomyosins, including our results, are aligned in **Figure 2**. It is worth mentioning that three types of tropomyosin (fast, slow-twitch, and slow-tonic) have been identified in American lobster (*14*, *15*). As compared to the amino acid sequence of the fast tropomyosin, both slow-twitch and slow-tonic tropomyosins have as many as 15 alterations in the region 39−79 and the slow-tonic tropomyosin has 4 additional alterations in the C-terminal region. On the basis of the sequence features of the three types of tropomyosin from American lobster, the tropomyosins from the abdominal muscle of shrimps (or prawns) and spiny lobster and one tropomyosin isoform from the chest protection muscle of king crab are assignable to the fast type, that from the leg muscle of horsehair crab to the slow-twitch type, and another tropomyosin isoform from the chest protection muscle of king crab, those from the leg and chest protection muscles of snow crab, and that from the chest protection muscle of horsehair crab to the slow-tonic type. The abdominal muscle tropomyosin of pink shrimp has some differences in the region 44−72 compared to the fast-type tropomyosin but is distinguishable from the slow-type tropomyosin. Thus, we tentatively classify the pink shrimp tropomyosin into the fast-type family. In the case of red crab tropomyosin, it is apparently a member of the slow-twitch or slow-tonic type family, although its C-terminal sequence is unclear. Taken together, the relationship

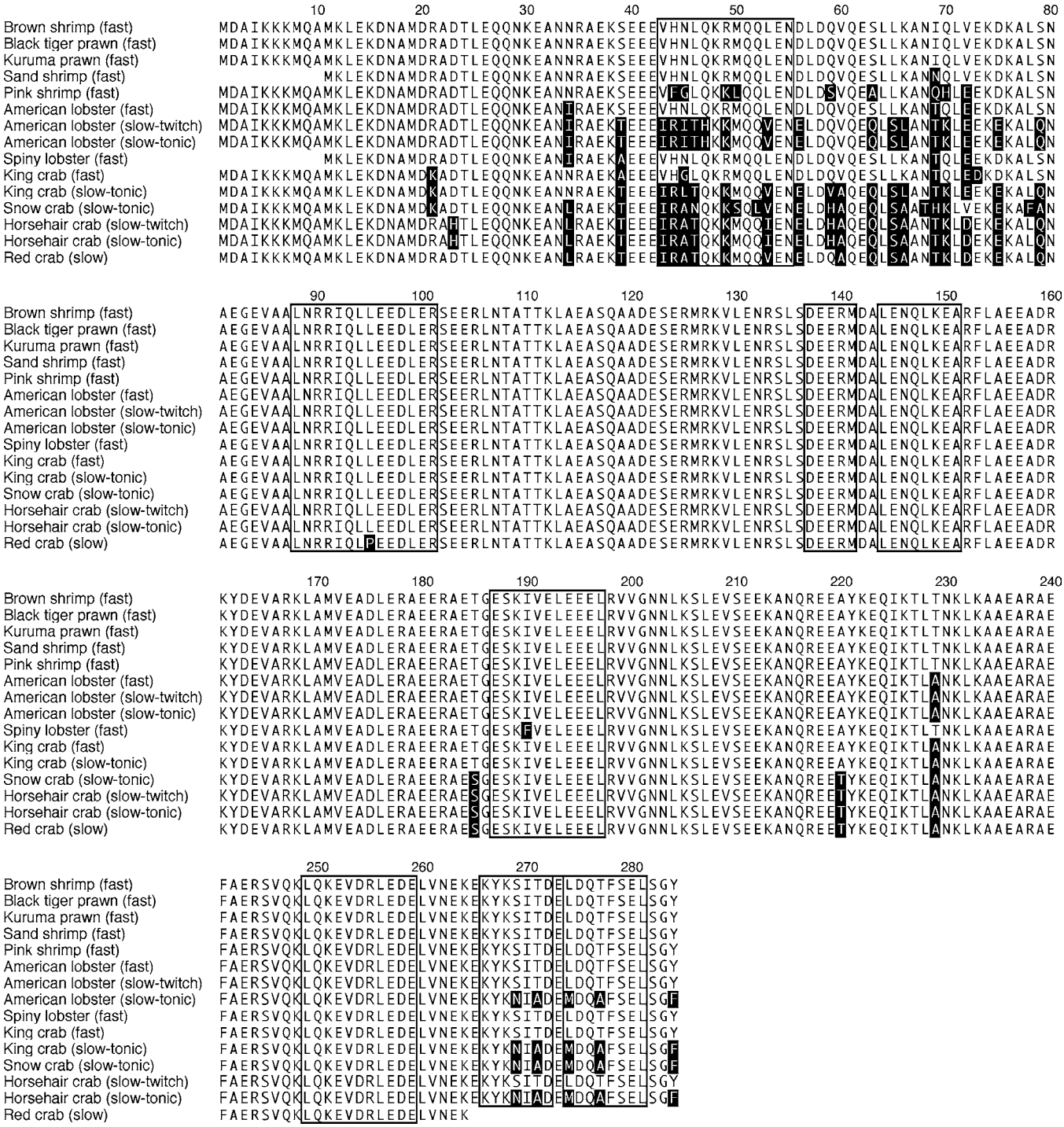


**Figure 2.** Amino acid sequence alignment of crustacean tropomyosins. Tropomyosin type (fast, slow-twitch, or slow-tonic) is indicated in parentheses after the crustacean name. Accession numbers (DDBJ/EMBL/GenBank nucleotide sequence databases): brown shrimp (*Penaeus aztecus*), DQ151457; black tiger prawn (*Penaeus monodon*), AB270629; kuruma prawn (*Penaeus japonicus*), AB270630; sand shrimp (*Metapenaeus ensis*), U08008; pink shrimp (*Pandalus eous*), AB270631; American lobster (*Homarus americanus*) (fast tropomyosin), AF034954; American lobster (slow-twitch tropomyosin), AF034953; American lobster (slow-tonic tropomyosin), AY521627; spiny lobster (*Panulinus stimpsoni*), AF030063; king crab (*Paralithodes camtschaticus*) (fast tropomyosin), AB270632; king crab (slow-tonic tropomyosins), AB270633; snow crab (*Chionoecetes opilio*), AB270634; horsehair crab (*Erimacrus isenbekii*) (slow-twitch tropomyosin), AB270635; horsehair crab (slow-tonic tropomyosin), AB270636; red crab (*Charybdis feriatus*), AF061783. The residues differing from the brown shrimp tropomyosin are shown in white on the black background. The IgE-binding epitopes proposed for the brown shrimp tropomyosin are boxed.

of the major tropomyosin type in edible muscles with the taxonomical position of crustaceans is summarized as follows: fast-type tropomyosins are contained in shrimps (or prawns) and lobsters, slow-type tropomyosins in crabs, and both fast- and slow-type tropomyosins in crayfishes and hermit crabs.

As shown in **Table 2**, the amino acid sequence identities between fast tropomyosins and between slow tropomyosins are extremely high; even 100% identity is observed among the fast tropomyosins from three species of *Penaeus* shrimp or prawn (brown shrimp, black tiger prawn, and kuruma prawn). The

**Table 2.** Amino Acid Sequence Identity between Crustacean Tropomyosins

| relationship | amino acid sequence identity (%) | |
|---|---|---|
| | range | mean ± SD |
| between fast tropomyosins | 96.0–100.0 | 98.1 ± 1.0 ($n$ = 28) |
| between slow tropomyosins | 92.3–98.5 | 96.1 ± 1.4 ($n$ = 21) |
| between fast and slow tropomyosins | 88.3–94.7 | 91.4 ± 1.4 ($n$ = 56) |

sequence identity between fast and slow tropomyosins is also considerably high. These facts seem to provide a molecular basis for the cross-reactivity among crustacean tropomyosins (*6*, *7*, *11*, *12*). More detailed discussion on the cross-reactivity among crustacean tropomyosins can be derived on the basis of the IgE-binding epitopes proposed for the brown shrimp tropomyosin (Pen a 1) (*10*, *16*). All eight epitope regions (43−55, 88−101, 137−141, 144−151, 187−197, 249−259, 266−273, and 273−281; refer to **Figure 2**) of Pen a 1 are well conserved in fast tropomyosins, with the exceptions of pink shrimp tropomyosin having four alterations in the region 43−55, spiny lobster tropomyosin having one alteration in the region 187−197, and king crab tropomyosin having one alteration in the region 43−55. This implies that the cross-reactivity among fast tropomyosins is simply realized by the common IgE-binding epitopes. In the case of slow tropomyosins, five of the eight Pen a 1 epitopes are completely or almost completely conserved. However, both slow-twitch and slow-tonic tropomyosins have as many as six to eight alterations in the region 43−55, and slow-tonic tropomyosins have additional two alterations in the two regions 266−277 and 273−281. Moreover, diverse alterations between fast and slow tropomyosins are also observed in the region 56−79, which is not identified as the Pen a 1 epitope. For a better understanding of the cross-reactivity between fast and slow tropomyosins, an examination of how the significant differences in specific regions between fast and slow tropomyosins are associated with the IgE-binding potency is needed.

In general, crustacean-allergic patients are mostly sensitive to both shrimp and crab. This is easily understood by the presence of several common IgE-binding epitopes in shrimp and crab tropomyosins. However, some crustacean-allergic patients specifically recognize only shrimp or crab. This can be realized by two possibilities. One is that both shrimp and crab have specific allergens differing from tropomyosins; for example, arginine kinase has recently been identified as a new allergen in black tiger prawn (*21*). Another possibility is derived from our finding that there are significant differences in amino acid sequences of specific regions between fast tropomyosins (mainly contained in shrimp) and slow tropomyosins (mainly contained in crab). Namely, it is possible that both shrimp-allergic and crab-allergic patients have IgE specifically binding to the regions with significant differences between fast and slow tropomyosins. In conclusion, the amino acid sequences of crustacean tropomyosins determined in this study will facilitate future molecular study to understand crustacean allergy in more detail.

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