

Sarcoplasmic Calcium-Binding Protein: Identification as a New Allergen of the Black Tiger Shrimp *Penaeus monodon*

Kazuo Shiomi Yuichiro Sato Shohei Hamamoto Hajime Mita
Kuniyoshi Shimakura

Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Tokyo, Japan

Key Words

Arginine kinase • Black tiger shrimp • *Penaeus monodon* • Sarcoplasmic calcium-binding protein • Tropomyosin

Abstract

Background: Tropomyosin and arginine kinase have been identified as crustacean allergens. During purification of arginine kinase from black tiger shrimp *Penaeus monodon*, we found a new allergen of 20-kDa. **Methods:** A 20-kDa allergen was purified from the abdominal muscle of black tiger shrimp by salting-out, anion-exchange HPLC and reverse-phase HPLC. Following digestion of the 20-kDa allergen with lysyl endopeptidase, peptide fragments were isolated by reverse-phase HPLC, and 2 of them were sequenced. The 20-kDa allergen, together with tropomyosin and arginine kinase purified from black tiger shrimp, was evaluated for IgE reactivity by ELISA. Five species of crustaceans (kuruma shrimp, American lobster, pink shrimp, king crab and snow crab) were surveyed for the 20-kDa allergen by immunoblotting. **Results:** The 20-kDa allergen was purified from black tiger shrimp and identified as a sarcoplasmic calcium-binding protein (SCP) based on the determined amino acid sequences of 2 enzymatic fragments. Of 16 sera from crustacean-allergic patients, 8 and 13 reacted to SCP and tropomyosin, respectively; the reactivity to arginine kinase was

weakly recognized with 10 sera. In immunoblotting, an IgE-reactive 20-kDa protein was also detected in kuruma shrimp, American lobster and pink shrimp but not in 2 species of crab. Preadsorption of the sera with black tiger shrimp SCP abolished the IgE reactivity of the 20-kDa protein, suggesting the 20-kDa protein to be an SCP. **Conclusions:** SCP is a new crustacean allergen, and distribution of IgE-reactive SCP is probably limited to shrimp and crayfish.

Copyright © 2008 S. Karger AG, Basel

Introduction

Crustaceans are widely consumed as delicious foods but are simultaneously recognized as one of the most common causes of IgE-mediated food allergy. Immediately after ingestion of crustaceans, sensitized individuals develop adverse reactions such as urticaria, asthma, diarrhea and even life-threatening anaphylaxis. The most extensively studied crustacean allergen is tropomyosin, a myofibrillar protein of 35–38 kDa constituting thin myofibrils together with actin and troponin. So far, tropomyosin has been established at the molecular level as the major allergen of a number of crustaceans including shrimp [1–4], crayfish [5], lobster [5], hermit crab [4], crab [4, 6] and barnacle [7]. In addition, tropomyosin has also

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2008 S. Karger AG, Basel
1018–2438/08/1462–0091\$24.50/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. Kazuo Shiomi
Department of Food Science and Technology
Tokyo University of Marine Science and Technology
Konan-4, Minato-ku, Tokyo 108-8477 (Japan)
Tel. +81 3 5463 0601, Fax +81 3 5463 0669, E-Mail shiomi@kaiyodai.ac.jp

been identified as the major allergen of mollusks such as cephalopods [8–10], gastropods [11, 12] and bivalves [12] and as one of the allergens of house dust mites [13, 14] and cockroaches [15, 16]. Thus, it is currently accepted that tropomyosin is a cross-reactive panallergen of invertebrates [15–20].

Besides tropomyosin, arginine kinase with a molecular mass of 40 kDa, which catalyzes the transfer of the high-energy phosphoryl group from ATP to arginine or inversely from N-phosphoarginine to ADP, was recently demonstrated to be a new but minor allergen of black tiger shrimp *Penaeus monodon* by proteomic analysis [21]. Inhibition studies showed the cross-reactivity of the black tiger shrimp arginine kinase with those from various species of crustaceans. Arginine kinase has previously been described as an allergen of the moth *Plodia interpunctella* and suggested to be cross-reactive among invertebrates including crustaceans [22].

To obtain a standardized crustacean allergen, we recently purified arginine kinase from black tiger shrimp by salting-out (70–90% saturation of ammonium sulfate) and anion-exchange HPLC, essentially according to the method of Yu et al. [21]. However, with anion-exchange HPLC, a 20-kDa IgE-binding protein differing from tropomyosin and arginine kinase was newly detected. This study was initiated to purify and identify the 20-kDa allergen detected in black tiger shrimp. We report here that the 20-kDa allergen is a sarcoplasmic calcium-binding protein (SCP) and that the distribution of IgE-reactive SCPs is probably limited to shrimp and crayfish.

Materials and Methods

Crustacean Samples

Fresh specimens of black tiger shrimp, kuruma shrimp *Penaeus japonicus*, American lobster *Homarus americanus*, pink shrimp *Pandalus eous*, king crab *Paralithodes camtschaticus* and snow crab *Chionoecetes opilio* were purchased at the Tokyo Central Wholesale Market. Abdominal muscle was obtained from each specimen of black tiger shrimp, kuruma shrimp, American lobster and pink shrimp and leg muscle from king crab and snow crab. Muscle samples were stored at –20°C until use.

Preparation of Crude Extract

Each muscle sample (2 g) was homogenized with 4 volumes of 0.6 M KCl in 10 mM phosphate buffer (pH 7.0) and centrifuged at 18,000 g for 20 min at 4°C. The supernatant obtained was used as a crude extract.

Purification of Tropomyosin from Black Tiger Shrimp

An acetone powder of myofibrillar proteins was prepared from the abdominal muscle of black tiger shrimp as reported by

Greaser and Gergely [23] and extracted twice with 2 volumes of 25 mM Tris-HCl buffer (pH 8.0) containing 1 M KCl, 0.1 mM CaCl₂ and 1 mM dithiothreitol (DTT). The extract was subjected successively to salting-out with ammonium sulfate (50% saturation) and isoelectrical precipitation (pH 4.6), essentially by the method of Cummins and Perry [24]. To remove small amounts of contaminated proteins, the tropomyosin-rich fraction thus obtained was further purified by application to reverse-phase HPLC on a TSK-gel 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 (TFA). The homogeneity of the final preparation was demonstrated by SDS-PAGE.

Purification of Arginine Kinase and a 20-kDa Allergen from Black Tiger Shrimp

The abdominal muscle (10 g) of black tiger shrimp was homogenized with 10 volumes of 0.1 M Tris-HCl buffer (pH 8.0) containing 0.025 mM phenylmethylsulfonyl fluoride, 1 mM DTT and 1 mM EDTA and centrifuged at 18,000 g for 10 min at 4°C. The supernatant was adjusted to 70% saturation with ammonium sulfate. After centrifugation, ammonium sulfate was added to the supernatant to give a final concentration of 90% saturation. The precipitate was collected by centrifugation, dissolved in 5 ml of 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM DTT and 1 mM EDTA and dialyzed against the same buffer. The dialysate was subjected to anion-exchange HPLC on a Mono Q HR5/5 column (0.5 × 5 cm; GE Healthcare Bio-Sciences, Piscataway, N.J., USA), which was eluted at a flow rate of 0.5 ml/min with a linear gradient of NaCl (0.0–0.5 M in 60 min) in 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM DTT and 1 mM EDTA. Proteins were monitored at 280 nm with a UV detector. In a preliminary run, fractions of 0.5 ml were collected and examined for arginine kinase activity and IgE reactivity to know the elution positions of arginine kinase and the 20-kDa allergen. Arginine kinase was obtained in a pure state in the eluate showing arginine kinase activity. On the other hand, the eluate exhibiting IgE reactivity was found to contain small amounts of impurities other than the 20-kDa allergen and was further applied to reverse-phase HPLC on a TSKgel ODS-120T column, which was eluted with a linear gradient of acetonitrile (0–56% in 60 min) in 0.1% TFA at a flow rate of 1 ml/min. Proteins were monitored at 220 nm with a UV detector. Thus, a pure preparation of the 20-kDa allergen was finally obtained.

Assay of Arginine Kinase Activity

Arginine kinase activity was assayed in the forward direction with a coupled enzyme system. The reaction mixture (1 ml) contained 0.05 ml of sample solution and the following reagents: 10 mM arginine, 37.5 mM KCl, 12.5 mM magnesium acetate, 1.25 mM phosphoenolpyruvate, 0.25 mM NADH, 5 mM ATP, 65 mM Tris-HCl (pH 8.0), 22.5 units of pyruvate kinase and 22.5 units of lactate dehydrogenase. The oxidation of NADH was measured at 25°C by monitoring the absorbance decrease at 340 nm. One unit of arginine kinase was defined as the amount of enzyme that releases 1 µmol NAD per minute.

Enzymatic Digestion and Isolation of Peptide Fragments

The purified 20-kDa allergen (150 µg) was dissolved in 0.2 ml of 25 mM Tris-HCl buffer (pH 8.5) containing 1 mM EDTA and

8 M urea and kept at room temperature for 4 h. The solution was diluted with 0.6 ml of 25 mM Tris-HCl buffer (pH 8.5) containing 1 mM EDTA and digested with 1 µg of lysyl endopeptidase (EC 3.4.21.50; Wako Pure Chemicals, Osaka, Japan) at 37°C for 18 h. Peptide fragments were isolated by reverse-phase HPLC on TSK-gel ODS-120T under the same conditions as adopted for the purification of the 20-kDa allergen. Peptides were monitored at 220 nm with a UV detector.

Amino Acid Sequencing

Amino acid sequencing was performed by the automated Edman degradation method using a gas-phase protein sequencer (model 492; Applied Biosystems, Foster City, Calif., USA).

Human Sera

Sera were obtained from 16 crustacean-allergic patients with clinical histories of immediate hypersensitive reactions after ingestion of crustaceans. The patients were all diagnosed to be allergic to crustaceans based on the determined CAP-RAST classes 2–5 against shrimp or both shrimp and crab. Written informed consent was obtained from each patient. In this study, sera from 14 healthy volunteers without adverse reactions after ingestion of any foods were used as controls. All sera were stored at –20°C until use.

Fluorescence ELISA

Fluorescence ELISA was performed as reported previously [25]. In brief, the antigen coated on a polystyrene microtiter plate with 96 wells (Type H, black; Sumitomo Bakelite, Tokyo, Japan) was reacted successively with patient or control serum (diluted 1:200) and β-galactosidase-conjugated goat anti-human IgE antibody (0.25 µg/ml; American Qualex, San Clemente, Calif., USA). Enzyme reaction was carried out using 0.1 mg/ml 4-methylumbelliferyl-β-D-galactoside and stopped by addition of 0.1 M glycine-NaOH buffer (pH 10.3). Fluorescence intensity was measured at 367 nm (excitation) and 453 nm (emission).

SDS-PAGE Analysis

SDS-PAGE was performed with a PhastSystem (GE Healthcare Bio-Sciences) according to the manufacturer's instructions. Each sample was dissolved in 62.5 mM phosphate buffer (pH 7.5) containing 2.5% SDS and 5% DTT, heated at 100°C for 5 min and subjected to electrophoresis on a PhastGel Gradient 8–25 gel (GE Healthcare Bio-Sciences). Precision Plus Protein Standards (Bio-Rad Laboratories, Hercules, Calif., USA) were run as a reference, along with samples. After running, proteins were stained with Coomassie Brilliant Blue R-250.

Immunoblotting

IgE immunoblotting was carried out as reported previously [10]. Briefly, the proteins separated by SDS-PAGE were electrotransferred from the gel to a nitrocellulose membrane (Hybond-ECL; GE Healthcare Bio-Sciences), which was reacted successively with patient serum (diluted 1:200) and horseradish peroxidase-conjugated goat anti-human IgE antibody (diluted 1:10,000; Kirkegaard & Perry Laboratories, Gaithersburg, Md., USA). Blots were visualized using ECL Plus Western Blotting Detection Reagents (GE Healthcare Bio-Sciences) according to the manufacturer's instructions. For inhibition IgE immunoblotting, patient

serum (diluted 1:100) was incubated with an equal volume of the 20-kDa allergen solution (20 µg/ml) at 37°C for 1 h and used as a primary antibody.

Results

Purification of Arginine Kinase and the 20-kDa Allergen from Black Tiger Shrimp

Arginine kinase of black tiger shrimp has previously been purified by salting-out (70–90% saturation of ammonium sulfate) and anion-exchange HPLC on HiTrap Q Sepharose [21]. In this study, the precipitate at 70–90% saturation of ammonium sulfate was similarly prepared from the crude extract of black tiger shrimp muscle and subjected to anion-exchange HPLC on a Mono Q column. As a result, arginine kinase was eluted in the first peak at a retention time of 25 min from the Mono Q column (fig. 1a), similar to the previous study, and its homogeneity was evidenced by SDS-PAGE (fig. 1c, lane 3). However, monitoring by ELISA using a serum pooled from 16 crustacean-allergic patients revealed that arginine kinase had no substantial IgE-binding ability. Instead, peak IgE reactivity was detected at a retention time of 42 min (fig. 1a). One major (20 kDa) and 1 minor (40 kDa) protein was contained in this peak (fig. 1c, lane 4) and only the 20-kDa protein was found to be IgE reactive by immunoblotting (data not shown). Finally, the 20-kDa allergen was purified by reverse-phase HPLC on TSKgel ODS-120T (fig. 1b; fig. 1c, lane 5). In a typical run, 20 mg of arginine kinase and 1 mg of the 20-kDa allergen were obtained from 10 g of the muscle. Moreover, the 20-kDa allergen could be readily purified by applying the precipitate at 70–90% saturation of ammonium sulfate directly to reverse-phase HPLC (data not shown); in this case, 10 g of the muscle yielded as much as 32 mg of the 20-kDa allergen.

Identification of the 20-kDa Allergen as an SCP

Peptide fragments produced upon digestion of the 20-kDa allergen with lysyl endopeptidase were isolated by reverse-phase HPLC and 2 (peptides 1 and 2) of them were randomly selected and sequenced as shown in figure 2. When searched by the protein databases on the basis of the determined amino acid sequences of the 2 peptides, the 20-kDa allergen was found to be a member of the SCP family. The SCPs from 3 species of crustaceans, oriental shrimp *Penaeus orientalis* [26, 27], narrow-fingered crayfish *Astacus leptodactylus* [28] and red swamp crayfish *Procambarus clarkii* [29], have been elu-

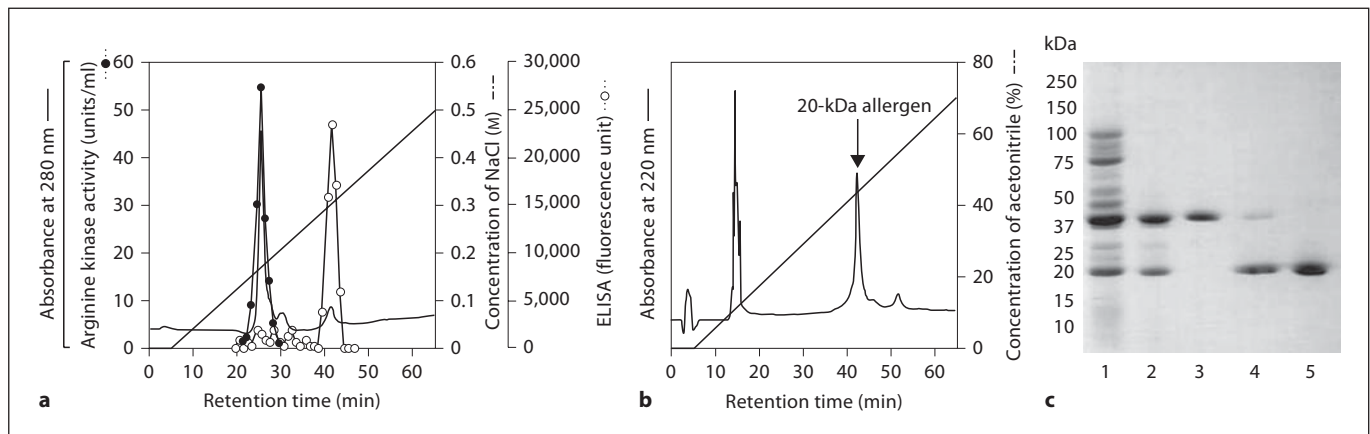


Fig. 1. Purification of arginine kinase and a 20-kDa allergen from the abdominal muscle of black tiger shrimp. **a** Anion-exchange HPLC. Sample, precipitate at 70–90% saturation of ammonium sulfate; column, Mono Q HR5/5 (0.5 × 5 cm); elution, linear gradient of NaCl in 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM DTT and 1 mM EDTA; flow rate, 0.5 ml/min. **b** Reverse-phase HPLC. Sample, IgE-reactive fraction obtained by anion-exchange HPLC; column, TSKgel ODS-120T (0.46 × 25 cm); elution, linear gradient of acetonitrile in 0.1% TFA; flow rate, 1 ml/min. **c** SDS-PAGE. Lane 1, crude extract; lane 2, precipitate at 70–90% saturation of ammonium sulfate; lane 3, arginine kinase fraction obtained by anion-exchange HPLC; lane 4, 20-kDa allergen fraction obtained by anion-exchange HPLC; lane 5, purified 20-kDa allergen obtained by reverse-phase HPLC.

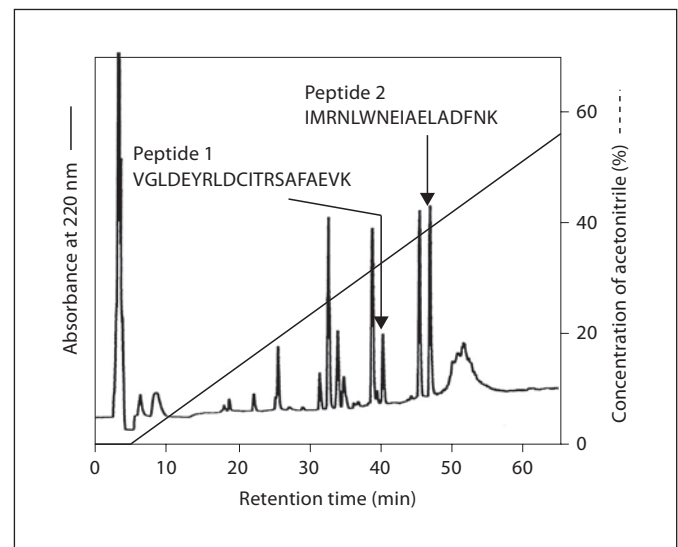


Fig. 2. Isolation of peptide fragments from the digest of the 20-kDa allergen with lysyl endopeptidase by reverse-phase HPLC. Refer to the legends of figure 1b for chromatographic conditions. The determined amino acid sequences of 2 peptides (peptides 1 and 2) are inserted in this figure.

cidated for their amino acid sequences, as shown in figure 3. Peptides 1 and 2 obviously correspond to the segments 120–139 and 56–72 of the crustacean SCPs, respectively. Particularly, peptide 1 is completely identical with the segment 120–139 of the oriental shrimp SCP α -A and α -B chains and peptide 2 with the segment 56–72 of the oriental shrimp SCP α -B chain.

Reactivity of Patient Sera to SCP, Tropomyosin and Arginine Kinase

IgE reactivity of SCP, tropomyosin and arginine kinase purified from black tiger shrimp was evaluated by ELISA using sera from 16 crustacean-allergic patients. As shown in figure 4, 8 sera reacted to SCP, 13 sera to tropomyosin and 10 sera to arginine kinase. Four sera (from

in kuruma shrimp, American lobster and pink shrimp is an SCP and that SCP is a cross-reactive allergen between shrimp and crayfish. From the staining intensity in SDS-PAGE (fig. 5a), the content of SCP was judged to be somewhat lower in kuruma shrimp, American lobster and pink shrimp than in black tiger shrimp. On the other hand, a 20-kDa protein corresponding to SCP was, if present, traced in the crude extracts from 2 species of crab as judged by SDS-PAGE. In accordance with this, no IgE-reactive 20-kDa protein was found in these crabs by immunoblotting (fig. 5b).

Discussion

Two classes of proteins, tropomyosin and arginine kinase, have so far been established to be major and minor allergens of crustaceans, respectively. In this study, the third crustacean allergen of 20 kDa was detected in black tiger shrimp during the purification procedure of arginine kinase and identified as SCP by the determination of its partial amino acid sequence.

Fig. 4. Analysis of IgE reactivity of SCP (a), tropomyosin (b) and arginine kinase (c) purified from black tiger shrimp by fluorescence ELISA using 16 patient sera. Control data (C) obtained with 14 healthy subjects were averaged out. The values above the control mean + 2 SD (indicated by a dotted line in each panel) were judged to be positive.

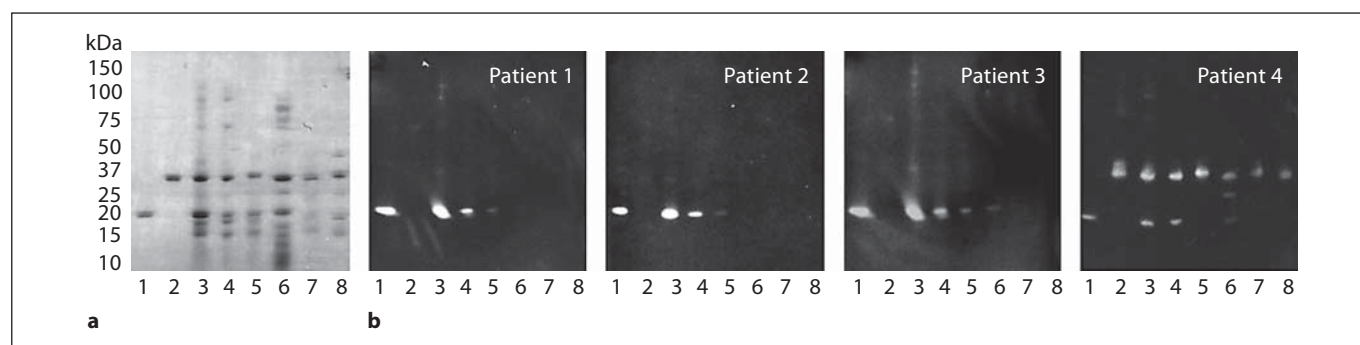
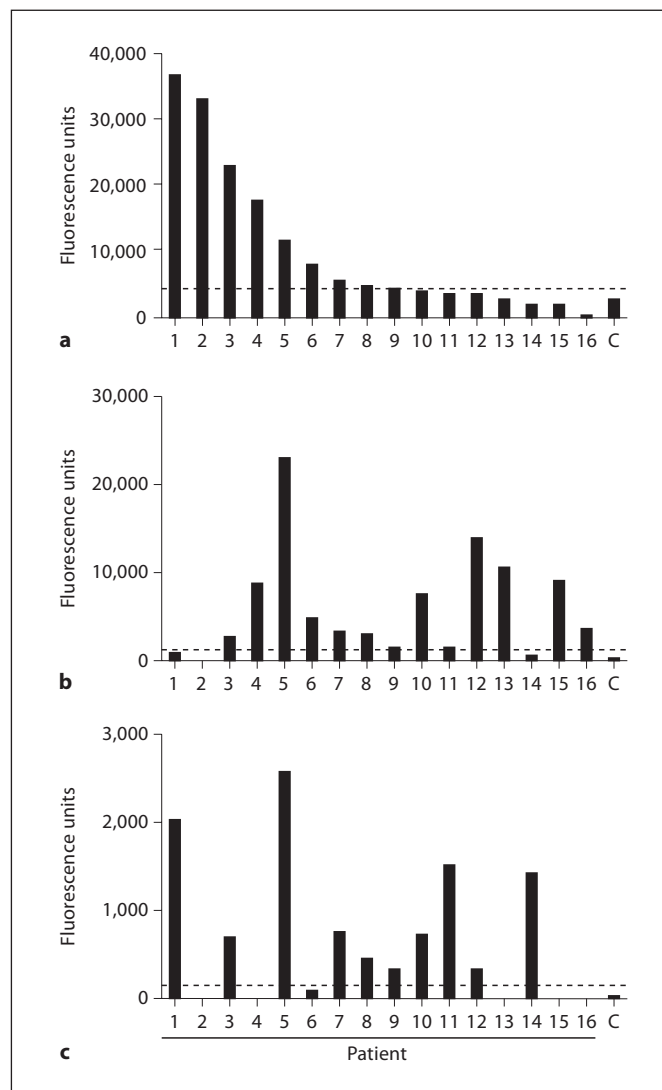


Fig. 5. Analysis of the crude extracts from crustaceans by SDS-PAGE (a) and immunoblotting using patient sera (b). Lane 1, SCP purified from black tiger shrimp; lane 2, tropomyosin purified from black tiger shrimp; lane 3, black tiger shrimp extract; lane 4, kuruma shrimp extract; lane 5, American lobster extract; lane 6, pink shrimp extract; lane 7, snow crab extract; lane 8, king crab extract.

In the previous study of Yu et al. [21], who purified a 40-kDa allergen from black tiger shrimp by salting-out (70–90% saturation of ammonium sulfate) and anion-exchange HPLC and confirmed it as arginine kinase, a 22-kDa protein (presumably identical with SCP), together with arginine kinase, was described as a major protein contained in the ammonium sulfate precipitate. However, focusing on arginine kinase, Yu et al. [21] estimated only arginine kinase activity in anion-exchange HPLC and were not aware of the presence of another allergen (SCP). In this study, both arginine kinase activity and IgE-binding ability were checked in anion-exchange HPLC, leading to the finding of SCP as the third crustacean allergen.

In ELISA experiments using the 3 allergens (SCP, tropomyosin and arginine kinase) purified from black tiger shrimp, as many as 13 of 16 patient sera were shown to react to tropomyosin, conforming well to the current opinion that tropomyosin is the major crustacean allergen [15, 18, 20]. SCP recognized by half the patient sera also seems to be a major allergen, although future study using much more patient sera is needed. While arginine kinase has previously been reported to be a minor allergen [21], it was recognized by 10 patient sera in this study. However, the IgE reactivity of arginine kinase was much less potent than those of tropomyosin and SCP. We thus assume that arginine kinase is of less importance in crustacean allergy than the other 2 allergens.

Crustacean-allergic patients are mostly sensitive to various species of crustaceans including shrimp, crayfish, lobster, hermit crab and crab. The cross-reactivity among crustaceans is attributable to the highly conserved amino acid sequences of crustacean tropomyosin molecules [4]. Arginine kinase may also be implicated to some extent in the cross-reactivity among crustaceans, since it is known to be a common allergen of crustaceans [21].

In contrast to the cross-reactivity described above, a part of crustacean-allergic patients are clinically known to be sensitive only to either shrimp or crab. There seem to be 2 possibilities. One possibility is that such patients have IgE recognizing regions peculiar only to shrimp or crab tropomyosins. Indeed, crustacean tropomyosins are divided into fast type mainly contained in shrimp and slow type (slow-twitch type and slow-tonic type) mainly contained in crab and there are significant differences in some specific regions between amino acid sequences of fast- and slow-type tropomyosins [4]. It is interesting to examine how such differences between fast and slow tropomyosins are associated with the IgE-binding potency. Another possibility is that shrimp and crab individu-

ally contain specific allergens. In relation to this, it is particularly worth mentioning that shrimp and crayfish contain considerable amounts of IgE-reactive SCPs, while crabs are devoid of or contain only trace amounts of IgE-reactive SCPs. One patient (patient No. 2), who recognized black tiger shrimp SCP but not black tiger tropomyosin and arginine kinase, may be sensitive to shrimp and crayfish but not to crab.

Although shrimp and crayfish SCPs are IgE reactive and also cross-reactive with each other, our immunoblotting data qualitatively suggest that SCPs from 2 species of *Penaeus* shrimp (*P. monodon* and *P. japonicus*) have higher IgE reactivity than those from American lobster and pink shrimp (*P. eous*). This seems to be derived from the significant differences in amino acid sequences of SCPs, as recognized between oriental shrimp (*Penaeus* shrimp) SCP and crayfish SCPs (fig. 3). In order to understand at the molecular level the cross-reactivity between crustacean SCPs, primary structures and IgE-binding epitopes of SCPs from various species of crustaceans should be elucidated.

Acknowledgements

We thank Dr. Yuki Hamada, Nagasaki University, for amino acid sequencing. This study was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan and a Grant-in-Aid for Research from the Ministry of Health, Labour and Welfare of Japan.

References

- 1 Shanti KN, Martin BM, Nagpal S, Metcalfe DD, Rao PV: Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. *J Immunol* 1993;151:5354–5363.
- 2 Daul CB, Slattery M, Reese G, Lehrer SB: Identification of the major brown shrimp (*Penaeus aztecus*) allergen as the muscle protein tropomyosin. *Int Arch Allergy Immunol* 1994;105:49–55.
- 3 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;94:882–890.
- 4 Motoyama K, Suma Y, Ishizaki S, Nagashima Y, Shiomi K: Molecular cloning of tropomyosins identified as allergens in six species of crustaceans. *J Agric Food Chem* 2007;55:985–991.

- 5 Leung PSC, Chen YS, Mykles DL, Chow WK, Li CP, Chu KH: Molecular identification of the lobster muscle protein tropomyosin, as a seafood allergen. *Mol Mar Biol Biotechnol* 1998;7:12–20.
- 6 Leung PSC, Chen YS, Gershwin ME, Wong SH, Kwan HS, Chu KH: Identification and molecular characterization of *Charybdis feriatus* tropomyosin, the major crab allergen. *J Allergy Clin Immunol* 1998;102:847–852.
- 7 Suma Y, Ishizaki S, Nagashima Y, Lu Y, Ushio H, Shiomi K: Comparative analysis of barnacle tropomyosin: divergence from decapod tropomyosins and role as a potential allergen. *Comp Biochem Physiol B Biochem Mol Biol* 2007;147:230–236.
- 8 Miyazawa H, Fukamachi H, Inagaki Y, Reese G, Daul CB, Lehrer SB, Inouye S, Sakaguchi M: Identification of the first major allergen of a squid (*Todarodes pacificus*). *J Allergy Clin Immunol* 1996;98:948–953.
- 9 Ishikawa M, Suzuki F, Ishida M, Nagashima Y, Shiomi K: Identification of tropomyosin as a major allergen in the octopus *Octopus vulgaris* and elucidation of its IgE-binding epitopes. *Fish Sci* 2001;67:934–942.
- 10 Motoyama K, Ishizaki S, Nagashima Y, Shiomi K: Cephalopod tropomyosins: identification as major allergens and molecular cloning. *Food Chem Toxicol* 2006;44:1997–2002.
- 11 Ishikawa M, Ishida M, Shimakura K, Nagashima Y, Shiomi K: Purification and IgE-binding epitopes of a major allergen in the gastropod *Turbo cornutus*. *Biosci Biotechnol Biochem* 1998;62:1337–1343.
- 12 Chuo KH, Wong SH, Leung PSC: Tropomyosin is the major mollusk allergen: reverse transcriptase polymerase chain reaction, expression and IgE reactivity. *Mar Biotechnol* 2000;2:499–509.
- 13 Aki T, Kodama T, Fujikawa A, Miura K, Shigeta S, Wada T, Jyo T, Murooka Y, Oka S, Ono K: Immunochemical characterization of recombinant and native tropomyosins as a new allergen from the house dust mite, *Dermatophagoides farinae*. *J Allergy Clin Immunol* 1995;96:74–83.
- 14 Yi FC, Cheong N, Shek PC, Wang DY, Chua KY, Lee BW: Identification of shared and unique immunoglobulin E epitopes of the highly conserved tropomyosins in *Blomia tropicalis* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2002;32:1203–1210.
- 15 Santos AB, Chapman MD, Aalberse RC, Vailes LD, Ferriani VP, Oliver C, Rizzo MC, Nasputz CK, Arruda LK: Cockroach allergens and asthma in Brazil: identification of tropomyosin as a major allergen with potential cross-reactivity with mite and shrimp allergens. *J Allergy Clin Immunol* 1999;104:329–337.
- 16 Asturias JA, Gomez-Bayon N, Arilla MC, Martinez A, Palacios R, Sanchez-Gascon F, Martinez J: Molecular characterization of American cockroach tropomyosin (*Periplaneta americana* allergen 7), a cross-reactive allergen. *J Immunol* 1999;162:4342–4348.
- 17 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;98:954–961.
- 18 Reese G, Ayuso R, Lehrer SB: Tropomyosin: an invertebrate pan-allergen. *Int Arch Allergy Immunol* 1999;119:247–258.
- 19 Ayuso R, Reese G, Leong-Kee S, Plante M, Lehrer SB: Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. *Int Arch Allergy Immunol* 2002;129:38–48.
- 20 Lehrer SB, Ayuso R, Reese G: Seafood allergy and allergens: a review. *Mar Biotechnol* 2003;5:339–348.
- 21 Yu CJ, Lin YF, Chiang BL, Chow LP: Proteomics and immunological analysis of a novel shrimp allergen, Pen m 2. *J Immunol* 2003;170:445–453.
- 22 Binder M, Mahler V, Hayek B, Sperr WR, Scholler M, Prozell S, Wiedermann G, Valent P, Valenta R, Duchene M: Molecular and immunological characterization of arginine kinase from the Indianmeal moth, *Plodia interpunctella*, a novel cross-reactive invertebrate pan-allergen. *J Immunol* 2001;167:5470–5477.
- 23 Greaser ML, Gergely J: Reconstitution of troponin activity from three protein components. *J Biol Chem* 1971;246:4226–4233.
- 24 Cummins P, Perry SV: The subunits and biological activity of polymorphic forms of tropomyosin. *Biochem J* 1973;133:765–777.
- 25 Hamada Y, Tanaka H, Sato A, Ishizaki S, Nagashima Y, Shiomi K: Expression and evaluation of IgE-binding capacity of recombinant Pacific mackerel parvalbumin. *Allergol Int* 2004;53:271–278.
- 26 Takagi T, Konishi K: Amino acid sequence of alpha chain of sarcoplasmic calcium binding protein obtained from shrimp tail muscle. *J Biochem* 1984;95:1603–1615.
- 27 Takagi T, Konishi K: Amino acid sequence of the beta chain of sarcoplasmic calcium binding protein (SCP) obtained from shrimp tail muscle. *J Biochem* 1984;96:59–67.
- 28 Jauregui-Adell J, Wnuk W, Cox JA: Complete amino acid sequence of the sarcoplasmic calcium-binding protein (SCP-I) from crayfish (*Astacus leptodactylus*). *FEBS Lett* 1989;243:209–212.
- 29 Gao Y, Gillen CM, Wheatly MG: Molecular characterization of the sarcoplasmic calcium-binding protein (SCP) from crayfish *Procambarus clarkii*. *Comp Biochem Physiol B Biochem Mol Biol* 2006;144:478–487.