

Reactivity of Shrimp Allergy-Related IgE Antibodies to Krill Tropomyosin

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Key Words

Krill • Tropomyosin • Allergy • IgE antibodies • RACE • Crustacea

Abstract

Background: Krill, which morphologically resembles small shrimp, represents small ocean crustaceans and has been used for human consumption in Japan and some other countries. The major allergen in crustaceans has been reported to be tropomyosin, but the allergenicity of krill tropomyosin remains uncertain. **Methods:** Amino acid sequences of tropomyosin in two species of krill (*Euphausia superba* and *E. pacifica*) were deduced. Recombinant krill tropomyosins were produced in *Escherichia coli* using a pCold IV vector system, and the cross-reactivity of shrimp allergy-related IgE to the recombinant tropomyosins and several animal protein extracts was assessed by immunoblotting. **Results:** The deduced amino acid sequences of the *E. superba* and *E. pacifica* tropomyosins (designated as Eup s 1 and Eup p 1, respectively) were 284 residues and showed significant homology to those of shrimp, lobster and crab tropomyosins. Shrimp allergy-related IgE reacted to approximately 38-kDa protein bands in krill (*E. superba*), shrimp, lobster and crab protein extracts but did not react to protein extracts from either mollusks or vertebrates. Furthermore, the IgE recognized rEup s 1 and rEup p 1 as 38-kDa protein bands, and absorption of the IgE with rEup s 1 removed IgE reactivity to recombinant tropomyosins and protein extracts from krill and shrimp. **Conclusions:** Krill tropomyosins included highly homologous sequences to previously reported IgE-binding ep-

itopes in Pen a 1 (tropomyosin of *Penaeus aztecus*). The cross-reactivity in shrimp allergy-related IgE binding among krill, shrimp, lobster and crab tropomyosins was revealed. These observations suggest the potential allergenicity of krill tropomyosin.

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Introduction

Crustacea, such as shrimp, lobster and crab, is a common cause of food allergies in adults. Crustacean allergy does not appear to remit easily, and the allergy reaction causes itching, hives, swelling of the lips and tongue, pulmonary symptoms, gastrointestinal symptoms and anaphylactic shock [1–3]. The major crustacean allergen, a heat-stable 34- to 38-kDa protein, has been reported to be tropomyosin which is associated with the thin filament in muscle and microfilaments in nonmuscle cells in all eukaryotic cells [3–7]. The tropomyosin in invertebrates is a highly conserved protein in amino acid sequence among a wide range of invertebrate animals and has been suggested to be a cross-reacting allergen; thus it is reported that some patients allergic to shrimp are allergic to other crustaceans and mollusks or nonedible insects [5, 6, 8–10]. On the other hand, the allergy reaction to tropomyosin in vertebrates is rare in humans, and the IgE from crustacean-allergic individuals does not generally bind to tropomyosins from vertebrates [11, 12]. Therefore, tropomyosin in invertebrates is thought to be a panallergen [3, 5]. Shrimp tropomyosins have been well documented

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among crustacean allergens [3, 5, 7, 13, 14], and the shrimp-specific IgE-binding regions in the brown shrimp Pen a 1 (tropomyosin of *Penaeus aztecus*) have also been identified using synthetic peptides and sera of 18 shrimp-allergic patients [12, 15, 16].

Krill, which are shrimp-like marine crustaceans, are euphausiids and belong to the Euphausiidae family. Antarctic krill, *Euphausia superba*, lives in the Antarctic Ocean, and the annual catch worldwide is about 100,000 tons (1995–2004) [17, 18]. Pacific krill, *E. pacifica*, lives in the Pacific Ocean, and is intensively fished in the sea around Japan with limited quantities (about 70,000 tons annually) [19]. In Japan, Russia, Korea and some other countries, krill is used not only for aquarium feeds and bait in fishing but also for many different kinds of processed foodstuffs (such as soups, Korean pickles and fish meals), seasoning material and material in the pharmaceutical industries. Although in a local area in the north-east Japan conventional belief has long warned that krill intake causes allergy symptoms in certain people, the allergenicity of krill itself remains uncertain. If the amino acid sequence of krill tropomyosin is significantly similar to that of allergic crustacean tropomyosin, krill and processed foods including krill derivatives may cause crustacean allergy.

The aim of this study was to clarify the allergenicity of krill tropomyosin through the application of a molecular biology technique. To achieve this aim, we investigated the amino acid sequence identity of tropomyosin between krill and shrimp through deducing amino acid sequences of the krill tropomyosins from their full-length cDNA and examined the cross-reactivity in shrimp allergy-related sera among krill, shrimp, lobster and crab protein extracts by immunoblotting. This study is the first report describing the potential allergenicity of krill tropomyosin.

Materials and Methods

Preparation of Protein Extracts from Organisms

Fresh samples of northern pink shrimp (*Pandalus eous*), Australian spiny lobster (*Panulirus cygnus*), Japanese common squid (*Todarodes pacificus*), Pacific abalone (*Haliotis discus*), common orient clam (*Meretrix lusoria*) and chum salmon (*Oncorhynchus keta*) were purchased from local supermarkets in Japan. Frozen samples of Antarctic krill (*E. superba*) and Pacific krill (*E. pacifica*) were obtained from a local fishing shop. Frozen samples of black tiger prawn (*Penaeus monodon*), red king crab (*Paralithodes camtschaticus*) and frozen sample of boiled tanner crab (*Chionoecetes bairdi*) were purchased from a local supermarket. Commonly edible parts of muscles were collected from crustaceans,

mollusks and fish. The muscle was boiled in a protein extraction buffer (20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 0.004% β -mercaptoethanol) for 10 min, and the boiled muscle was then homogenized with a MultiBeads Shocker (Yasui Kikai, Osaka, Japan) at 2,500 rpm for 30 s. After centrifuging the homogenate at 13,000 g for 10 min, the supernatant was aliquoted and stored at -80°C until use for immunoblotting. The protein concentration in each soluble muscle protein solution was determined using a protein assay kit (Bio-Rad Laboratories, Hercules, Calif., USA) with bovine serum albumin (BSA) as the standard. All procedures were performed according to the manufacturer's protocols.

Deduced Amino Acid Sequences of Krill Tropomyosins

Total RNA was isolated from the muscles of the frozen Antarctic krill and Pacific krill with the use of an RNeasy total RNA isolation kit (Qiagen, Hilden, Germany), and then treated with an RNase-free DNase (Qiagen) to eliminate potentially contaminating DNA.

Tropomyosin cDNA in two species of krill was cloned using a combination of RT-PCR and the RNA ligase-mediated rapid amplification of the 5'- and 3'-cDNA ends (RLM-RACE). The partial cDNA sequences were determined by sequencing of the PCR products (833 bp) amplified from krill cDNA (synthesized from total RNA using a Qiagen QuantiTect Reverse Transcription kit) with two different PCR primers (5'-GGACGCCATCAAGAAG-AAGATG-3' and 5'-TGAAAGTCTGGTCCAGCTCGTC-3') designed based upon consensus sequences among the sand shrimp (*Metapenaeus ensis*, GenBank accession No. MEU08008), the black tiger prawn (*P. monodon*, AF253947), the American lobster (*Homarus americanus*, AF034954) and the red crab (*Charybdis feriatus*, AF061783) tropomyosin cDNA sequences. These partial cDNA sequences were used to design a forward and reverse gene-specific internal primer (5'-GGACGCCATCAAGAAGAAGATG-3' and 5'-CAGACACTTCCAGGGATTTGAGG-3', respectively) on performing RLM-RACE. RLM-RACE was carried out with a GeneRacer kit (Invitrogen, Carlsbad, Calif., USA) using total RNA.

The resultant PCR-RACE product was ligated into a TA vector (Invitrogen) and was sequenced using Applied Biosystems 3130. The full-length cDNA sequences of two different krill (*E. superba* and *E. pacifica*) tropomyosins were obtained from the partial cDNA, 5'-cDNA and 3'-cDNA sequences and were uploaded in the DDBJ/EMBL/GenBank databases under the accession No. AB289603 and AB289604, respectively. Amino acid sequences of *E. superba* and *E. pacifica* tropomyosins were deduced from the full-length cDNA sequences, and we named them Eup s 1 and Eup p 1, respectively, according to the guidelines of the International Union of Immunological Societies Allergen Nomenclature Subcommittee [20].

Preparation of Recombinant Krill Tropomyosin

The pCold protein production system in *Escherichia coli* [21, 22] was utilized to express the krill tropomyosin. The full-length cDNA fragment was obtained from the PCR with KOD-Plus-DNA polymerase (TOYOBO, Osaka, Japan) using KT-NdeI-S primer (5'-AGTCACATATGGACGCCATCAAGAAGAAGATGC-3') and KT-BamHI-AS primer (5'-CTGAAGGATCCTTAATATCCAGAGAGCTCGTTGAAGGTC-3') deduced from the two different krill tropomyosin cDNA sequences obtained. The NdeI-BamHI fragment (857 bp) was inserted into the NdeI-

*Bam*HI sites of pCold IV vector (accession No. AB186391), and expanded in *E. coli* strain TOP10F' (Invitrogen). The recombinant plasmid DNA sequence was confirmed by DNA sequencing.

For the purpose of expression, *E. coli* cells harboring the recombinant Antarctic krill (pCold IV rEup s 1) or Pacific krill (pCold IV rEup p 1) tropomyosin-encoding plasmid were grown at 35°C in Luria-Bertani medium containing 50 µg/ml of ampicillin with shaking. When the OD₆₀₀ reached 0.5, the cultures were shifted to a 15°C water bath and 0.1 mM isopropyl-β-D-thiogalactopyranoside was added to induce protein expression. After overnight incubation at 15°C with shaking, the cells were harvested by centrifugation and suspended in the protein extraction buffer. The cell suspension was boiled for 15 min and then centrifuged. The supernatant was stored at -80°C until use.

For the purification of the recombinant protein, the boiled cell suspension prepared from expression culture (500 ml) was clarified by centrifugation. The supernatant, passed sequentially through 3- and 0.45-µm Durapore filters, was applied to a Mono Q 16/10 HR anion exchange column (GE Healthcare, Amersham Biosciences AB, Uppsala, Sweden) preequilibrated with the protein extraction buffer. Fractions with the 38-kDa band in SDS-PAGE were eluted with a 250-ml linear gradient from 0–1 M NaCl in the same buffer. The fractions obtained were concentrated by ultrafiltration and exchanged into 20 mM Tris-HCl, pH 7.5, 5 mM EDTA buffer using a Centricon-3 (Millipore). The yield of the purified recombinant proteins was in the range of 38–40 mg/l.

Human Sera

Sera were obtained from 2 adult shrimp-allergic subjects (serum IgE levels: 190–1,600 IU/ml; RAST score to shrimp: 0.42–0.75 AU/ml) who had shown immediate hypersensitivity reactions (swelling of the lips, oropharyngeal pruritus or hives) after ingesting shrimp or crab. Strong IgE reactivity to shrimp protein was demonstrated in the sera of all the subjects by immunoblot analysis. A serum was also collected from a squid-allergic patient (hives; serum IgE level: 320 IU/ml; RAST score to shrimp: ≤0.34 AU/ml) who had no allergy to shrimp and crab. Sera from three adult normal volunteers (serum IgE levels: 23–110 IU/ml; RAST score to shrimp: ≤0.34 AU/ml) without any previous history of crustacean or other seafood allergy were used as controls. These sera were stored at -20°C until use. All subjects provided written informed consent prior to participation, and the local ethics committee approved the study.

SDS-PAGE and Immunoblotting

The protein sample (5–10 µg/lane) prepared according to the procedure of Laemmli [23] was resolved by SDS-PAGE on 12% separation gels under reducing conditions, and the resolved proteins were transferred onto a polyvinylidene difluoride membranes (Immobilon-PSQ, Millipore) using CAPS buffer as described by Matsudaira [24]. The membrane blocked with 3% BSA in Tris-buffered saline (TBS) was incubated in the test serum (1:15 dilution) in TBS with 3% BSA at 10°C for 2 days. After washing with TBS containing 0.1% Tween 20 4 times, the membrane was incubated with anti-human IgE goat-antibody-conjugated alkaline phosphatase (Sigma, St. Louis, Mo., USA; 1:2,000 dilution) in TBS with 3% BSA at 10°C for 1 day. After washing again, the blot was developed in a substrate solution of 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium for 10–15 min.

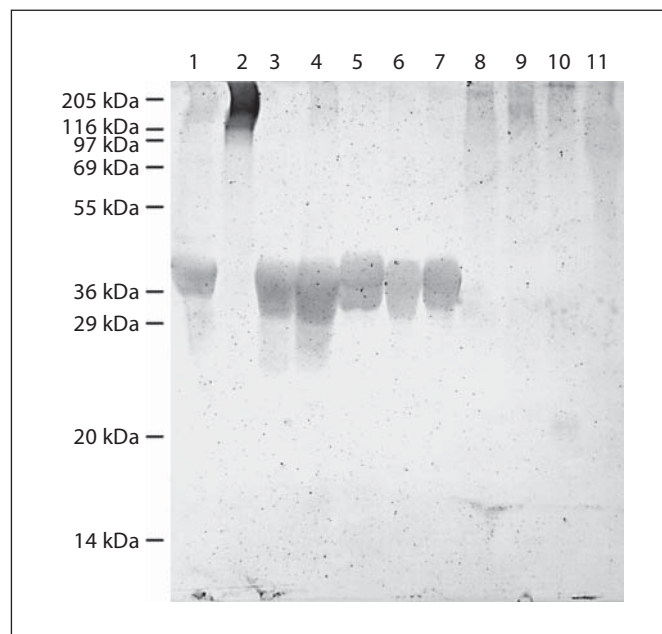


Fig. 1. Representative IgE immunoblotting of crustaceans, mollusks and fish muscle protein extracts with serum of a shrimp-sensitive subject. Lane 1: Antarctic krill *E. superba*; lane 2: Pacific krill *E. pacifica*; lane 3: black tiger prawn *P. monodon*; lane 4: northern pink shrimp *P. eous*; lane 5: Australian spiny lobster *P. cygnus*; lane 6: red king crab *P. camtschaticus*; lane 7: tanner crab *C. bairdi*; lane 8: Japanese common squid *T. pacificus*; lane 9: Pacific abalone *H. discus*; lane 10: common orient clam *M. lusoria*; lane 11: chum salmon *O. keta*. All samples were reduced prior to SDS-PAGE.

For the inhibition study of the IgE reactivity to recombinant tropomyosins and protein extracts from krill and shrimp by rEup s 1, the serum from a shrimp-allergic subject was diluted 1:15 in TBS with 3% BSA and incubated with purified rEup s 1 (approximately 2 mg/ml) at 10°C for 1 day. The preabsorbed serum was then used for the inhibition study.

Results

Reactivity of Shrimp-Sensitive Sera to Crustacean and Other Animal Muscle Protein Extracts

The boiled muscle protein extracts from crustaceans, mollusks and vertebrates were subjected to immunoblotting with sera from 2 shrimp-allergic subjects, 1 squid-allergic subject or 3 nonallergic subjects. The sera from 2 shrimp-allergic subjects reacted to approximately 38-kDa protein bands in krill (except for Pacific krill), shrimp, lobster and crab protein samples but did not react to the samples from either mollusk or vertebrate. These

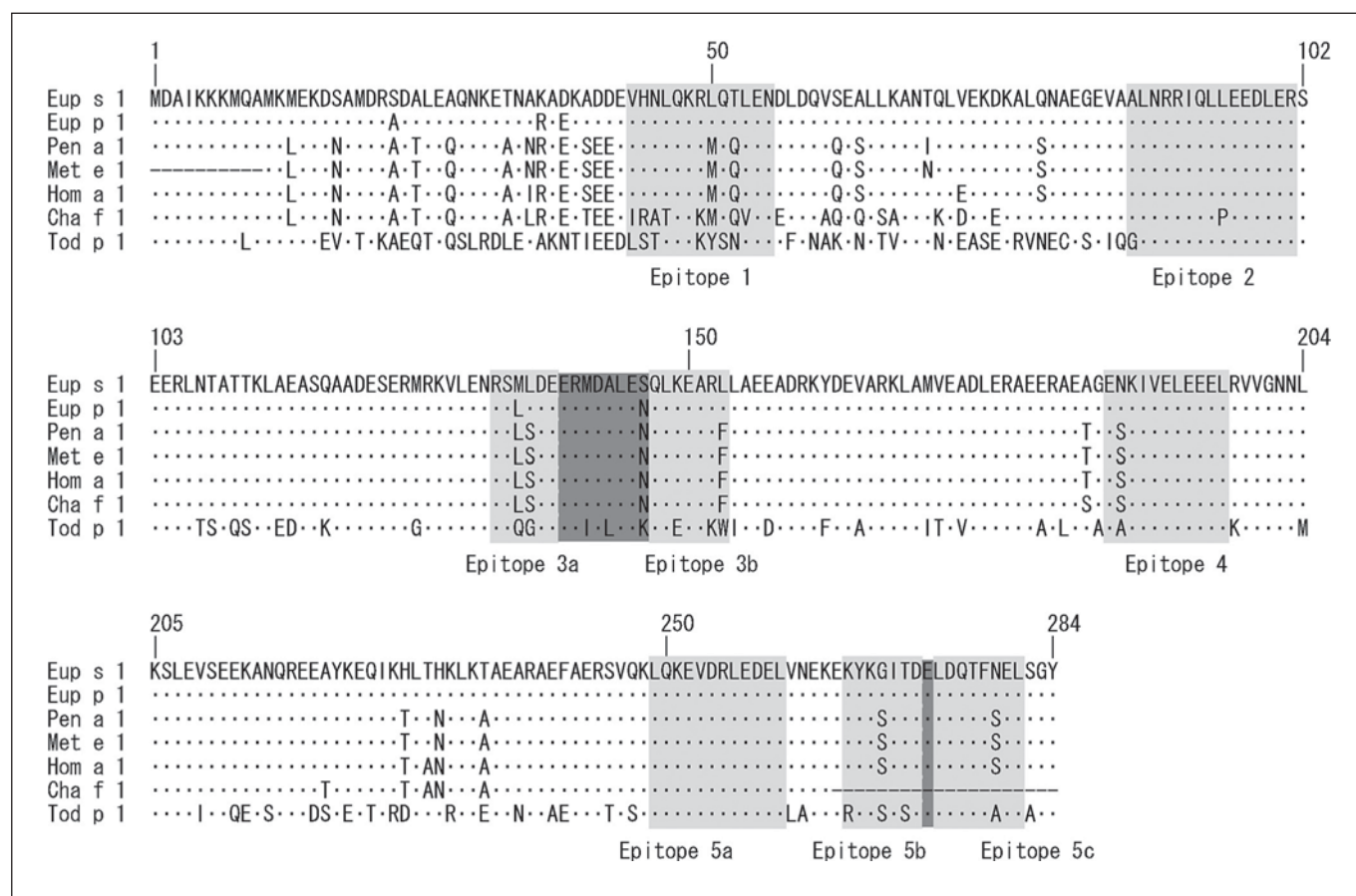


Fig. 2. Amino acid sequence alignments of krill, shrimp, lobster, crab and squid tropomyosins, and comparison of Pen a 1 IgE-binding epitopes [16] with homologous sequences in krill and other invertebrate tropomyosins. Eup s 1: tropomyosin from the krill *E. superba* (accession No. AB289603); Eup p 1: tropomyosin from krill *E. pacifica* (AB289604); Pen a 1: tropomyosin from brown shrimp *P. aztecus* (AAZ76743); Met e 1: tropomyosin from greasyback shrimp *M. ensis* (Q25456); Hom a 1: tropomyosin from American lobster *H. americanus* (AAC48288); Cha f 1: tropomyosin from crab *C. feriatius* (AAF35431); Tod p 1: tropomyosin from Japanese common squid *T. pacificus* (BAE54431). The shaded areas represent the epitopes. The densely shaded areas show the overlapping areas between two epitopes.

sera also reacted to multiple high-molecular-weight protein bands (>100 kDa) in Pacific krill sample. Representative IgE immunoblotting of these samples with the serum of a shrimp-allergic subject is shown in figure 1. One squid allergy-related serum reacted to an approximately 38-kDa protein band in a squid sample but did not react to any other samples (data not shown). Three normal sera hardly reacted with any of the samples used in this experiment (data not shown).

Sequence Analyses of the Eup s 1 and Eup p 1

The deduced amino acid sequences of Eup s 1 and Eup p 1 are shown in figure 2. Nucleotide sequence analysis of Eup s 1 and Eup p 1 cDNA showed an open reading

frame of 284 amino acids each. Thus Eup s 1 and Eup p 1 are the same length as shrimp (Pen a 1) and lobster (Hom a 1) tropomyosins. Amino acid sequence identity between Eup s 1 and Eup p 1 was 98%. The sequence identity of Eup s 1 with shrimp, lobster, crab and squid tropomyosins was 89–90, 89, 84 and 63%, respectively; thus the sequences of krill tropomyosins were highly similar to those of shrimp, lobster and crab tropomyosins.

Amino acid sequence comparison of eight major IgE-binding epitopes of Pen a 1, which had been characterized using sera of 18 shrimp-allergic subjects [12, 15, 16], with homologous sequences in two different krill and other invertebrate tropomyosins, is also shown in figure 2, and the shaded areas indicate the amino acid sequences homolo-

Table 1. Sequence identity (%) among Pen a 1 IgE-binding epitopes [16] and homologous sequences in krill, shrimp, lobster, crab and squid tropomyosins

Tropomyosin	Pen a 1 IgE-binding epitope							
	1	2	3a	3b	4	5a	5b	5c
Eup s 1	85	100	79	87	91	100	88	89
Eup p 1	85	100	86	93	91	100	88	89
Met e 1, Hom a 1	100	100	100	100	100	100	100	100
Cha f 1	54	93	100	100	100	100	NA	NA
Tod p 1	46	93	64	60	91	100	75	89

Values are expressed in percentage rounded off to the nearest whole number. Eup s 1: Tropomyosin from krill *E. superba* (accession No. AB289603); Eup p 1: tropomyosin from krill *E. pacifica* (AB289604); Pen a 1: tropomyosin from brown shrimp *P. aztecus* (AAZ76743); Met e 1: tropomyosin from greasyback shrimp *M. ensis* (Q25456); Hom a 1: tropomyosin from American lobster *H. americanus* (AAC48288); Cha f 1: tropomyosin from crab *C. feriatius* (AAF35431); Tod p 1: tropomyosin from Japanese common squid *T. pacificus* (BAE54431). NA = Cha f 1, which is shorter than Pen a 1 by 20 amino acid residues in the C-terminal tail, has no sequence region corresponding to the epitopes 5b and 5c.

gous to the Pen a 1 IgE-binding epitopes. The amino acid sequence identities of the epitopes with corresponding sequences in each tropomyosin are shown in table 1. A high degree of sequence identity was observed between Pen a 1 epitopes and homologous sequences in tropomyosins of two species of krill for all of the epitopes. Pen a 1 epitopes 2 (positions 87–101 in Pen a 1) and 5a (positions 249–260) had 100% identity with homologous sequence in Eup s 1 and Eup p 1. The epitopes 4 (positions 187–197), 5b (positions 266–273) and 5c (positions 273–281) differed in only one amino acid residue from those two sequences, resulting in sequence identities of 91, 88 and 89%, respectively. The epitopes 1 (positions 43–55), 3a (positions 133–146) and 3b (positions 139–153) differed at one, two or three amino acid positions from those sequences; the sequence identities were 85, 79–86 and 87–89%, respectively.

Reactivity of Shrimp-Sensitive Sera to Two Recombinant Krill Tropomyosins

Recombinant krill tropomyosins (rEup s 1 and rEup p 1) were overproduced as soluble and nonfusion proteins in *E. coli* using the pCold IV vector system based on the *cspA* promoter which is highly induced at low temperature [21, 22]; the proportion of the recombinant tropomyosins expressed in cells to total cell proteins was very high (fig. 3). Figure 3 shows results of cell fractionation and purification process on rEup s 1. The rEup s 1 accumulated in *E. coli* cells was recovered in the soluble fraction after cell disruption by heat treatment; and the soluble fraction contained two protein bands: major band of approximately 38 kDa, as expected for recombinant krill

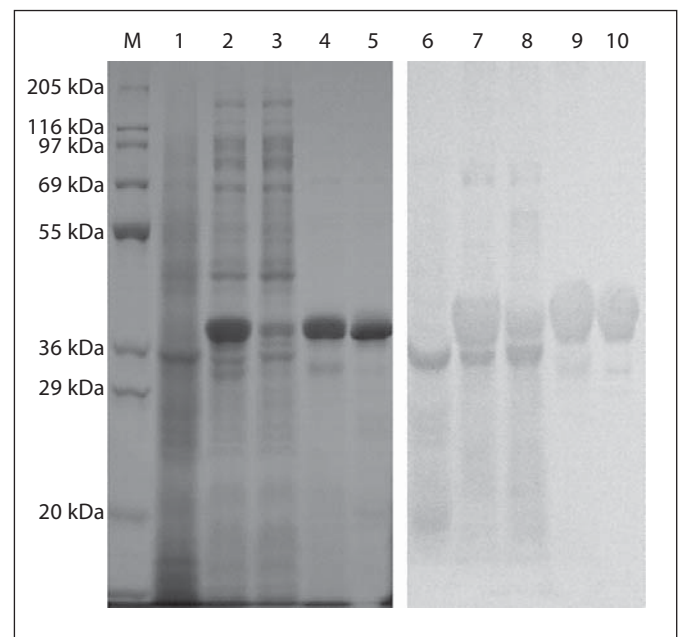


Fig. 3. SDS-PAGE analysis and IgE immunoblotting of rEup s 1 expressed in *E. coli*. Lanes M and 1–5: SDS-PAGE gel stained with SERVA Blue G (Serva, Heidelberg, Germany); lanes 6–10: IgE immunoblotting with serum of a shrimp-allergic subject. M: Molecular weight markers; lanes 1 and 6: IPTG-induced *E. coli* cells harboring pCold IV; lanes 2 and 7: IPTG-induced *E. coli* cells harboring pCold IV rEup s 1; lanes 3 and 8: insoluble cell fraction from the rEup s 1 expressed *E. coli* cells; lanes 4 and 9: soluble cell fraction from the rEup s 1 expressed *E. coli* cells; lanes 5 and 10: rEup s 1 preparation purified from the soluble cell fraction using anion exchange resins. All samples were reduced prior to SDS-PAGE.

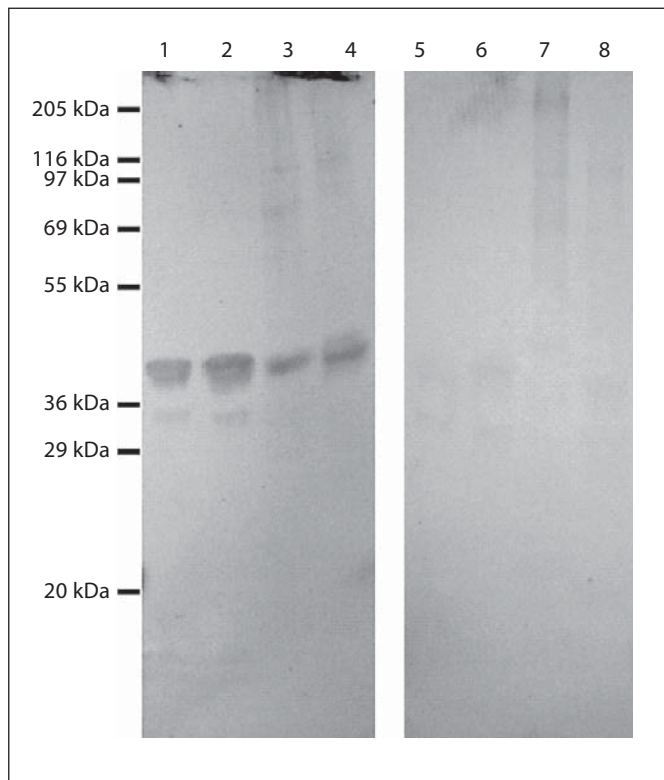


Fig. 4. Inhibition of IgE reactivity to recombinant krill tropomyosins and protein extracts from Antarctic krill and black tiger prawn by rEup s 1. Lanes 1–4: immunoblotting with unabsorbed serum; lanes 5–8: immunoblotting with serum absorbed by purified rEup s 1. Lanes 1 and 5: rEup s 1 soluble fraction; lanes 2 and 6: rEup p 1 soluble fraction; lanes 3 and 7: Antarctic krill protein extract; lanes 4 and 8: black tiger prawn protein extract.

tropomyosin, and minor band of approximately 34 kDa, probably as host-derived protein. The rEup s 1 and rEup p 1 were recognized as a 38-kDa band which is the same size as protein bands being detected in Atlantic krill and black tiger prawn protein extracts by immunoblotting with serum of a shrimp-allergic subject but not recognized in case of the use of the serum preabsorbed with the excess rEup s 1 which was purified from the soluble fraction (fig. 4). The preabsorbed serum also lost the IgE reactivity totally against 38-kDa protein bands in krill and shrimp protein extracts (fig. 4).

Discussion

The cross-reactivity of shrimp-specific IgE to other crustacean proteins has been well reported [3, 5, 10, 16], whereas the cross-reactivity of the IgE to krill protein has

not been known. In our study, we indicated that the IgE of the sera from shrimp-sensitive subjects are reactive with a 38-kDa band in muscle protein extracts not only in shrimp, lobster and crab but also in krill (fig. 1) and that the recombinant krill tropomyosins, rEup s 1 and rEup p 1, produced as nonfusion protein, are recognized as a 38-kDa band by immunoblotting (fig. 3). Moreover, preabsorption with purified rEup s 1 removed the IgE reactivity to recombinant tropomyosins and 38-kDa band in protein extracts from krill and shrimp (fig. 4). These results would reveal that IgE of the shrimp-allergic subjects cross-reacts with the krill tropomyosin as well as with other crustacean tropomyosins. However, a study of a great number of sera needs to be done to further ensure these results. IgE recognized multiple high-molecular-weight protein bands (>100 kDa) in Pacific krill protein extract and a 38-kDa band in the crude rEup p 1 sample (fig. 1, 4). Although the reason for this phenomenon remains to be solved, the formation of the multiple high-molecular-weight proteins may be due to unknown cross-linking between the tropomyosin and other proteins.

Furthermore, the high degree of sequence identity (79–100%) observed between the Pen a 1 epitopes and homologous sequences in krill tropomyosins for all of the epitopes (table 1) would support the cross-reactivity of the IgE to krill and other crustacean tropomyosins. This result suggests that krill tropomyosin is also the potential allergen in crustacean allergy and that food containing krill or its derivatives may cause crustacean allergy symptoms in shrimp-sensitive individuals. Clinical cases of allergy to krill have hardly been known. This may be due to mistaking krill for shrimp because krill morphologically resembles small shrimp. Therefore, further studies will be necessary to elucidate the clinical relevance of the potential allergenicity of krill.

In order to protect the health of consumers suffering from food allergies or intolerances, the Ministry of Health, Labour and Welfare in Japan has required mandatory labeling of five food allergen-related materials (egg, milk, wheat, buckwheat and peanut) on processed foods and has also recommended voluntary labeling for twenty other allergenic foods (abalone, squid, salmon roe, shrimp, crab, orange, kiwi fruit, beef, walnut, salmon, mackerel, soybean, chicken, pork, matsutake mushroom, peach, yam, apple, gelatin and banana); however, no voluntary labeling for krill and lobster has yet been recommended. Therefore, based on our results, the adding of krill and lobster to the voluntary labeling list in Japan would be helpful for shrimp-sensitive individuals.

Recombinant allergens are considered the basis for new diagnostic approaches and development of novel strategies of allergen-specific immunotherapy, and recombinant tropomyosins have often been produced as fusion proteins in *E. coli* [6, 7, 10, 12, 14, 25]. Although the characteristics of fused recombinant tropomyosins have been similar to those of the original tropomyosins [6, 7, 12], for the development of novel strategies of crustacean allergic-specific immunotherapy it would be preferable if they were produced as a nonfusion protein rather than any fusion protein. We showed that the nonfusion recombinant krill tropomyosin can be overproduced as a predominantly soluble and immunodetectable protein in an *E. coli* expression host by the pCold vector system [21, 22]

and that the expressed tropomyosins can be purified without any difficulty (fig. 3). Therefore, the pCold vector system would be advantageous in overproducing soluble and nonfusion recombinant tropomyosins in crustaceans at least for new diagnostic approaches and development of novel strategies of allergen-specific immunotherapy.

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References

- 1 Daul CB, Morgan JE, Lehrer SB: Hypersensitivity reactions to crustacea and mollusks. *Clin Rev Allergy* 1993;11:201–222.
- 2 Jeebhay MF, Robins TG, Lehrer SB, Lopata AL: Occupational seafood allergy. *Occup Environ Med* 2001;58:553–562.
- 3 Lehrer SB, Ayuso R, Reese G: Seafood allergy and allergens. *Mar Biotechnol* 2003;5:339–348.
- 4 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.
- 5 Reese G, Ayuso R, Lehrer SB: Tropomyosin: an invertebrate pan-allergen. *Int Arch Allergy Immunol* 1999;119:247–258.
- 6 DeWitt ÅM, Mattsson L, Lauer I, Reese G, Lidholm J: Recombinant tropomyosin from *Penaeus aztecus* (rPen a 1) for measurement of specific immunoglobulin E antibodies relevant in food allergy to crustaceans and other invertebrates. *Mol Nutr Food Res* 2004;48:370–379.
- 7 Reese G, Schickantz S, Lauer I, Randow S, Lüttkopf D, Vogel L, Lehrer SB, Vieths S: Structural, immunological and functional properties of natural recombinant Pen a 1, the major allergen of Brown Shrimp, *Penaeus aztecus*. *Clin Exp Allergy* 2006;36:517–524.
- 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 Leung PS, 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.
- 10 Leung PS, Chen YC, 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.
- 11 Ayuso R, Lehrer SB, Tanaka L, Ibañez MD, Pascual C, Burks AW, Sussman GL, Goldberg B, Lopez M, Reese G: IgE antibody response to vertebrate meat proteins including tropomyosin. *Ann Allergy Asthma Immunol* 1999;83:399–405.
- 12 Reese G, Viebranz J, Leong-Kee SM, Plante M, Lauer I, Randow S, Moncin MS, Ayuso R, Lehrer SB, Vieths S: Reduced allergenic potency of VR9-1, a mutant of the major shrimp allergen Pen a 1 (tropomyosin). *J Immunol* 2005;175:8354–8364.
- 13 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.
- 14 Leung PS, 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.
- 15 Ayuso R, Lehrer SB, Reese G: Identification of continuous, allergenic regions of the major shrimp allergen Pen a 1 (tropomyosin). *Int Arch Allergy Immunol* 2002;127:27–37.
- 16 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.
- 17 CCAMLR Statistical Bulletin (1996–2005). Hobart, CCAMLR, 2006, vol 18.
- 18 Naganobu M: Antarctic krill (*Euphausia superba*), the Antarctic ocean; in *The Current Status of International Fishery Stocks* in 2005. Tokyo, Fisheries Agency & Fisheries Research Agency, 2005, pp 424–432.
- 19 Nicol S, Foster J: Recent trends in the fishery for Antarctic krill. *Aquat Living Resour* 2003;16:42–45.
- 20 King TP, Hoffman D, Lowenstein H, Marsh DG, Platts-Mills TA, Thomas W: Allergen nomenclature. WHO/IUIS Allergen Nomenclature Subcommittee. *Int Arch Allergy Immunol* 1994;105:224–233.
- 21 Schein CH: A cool way to make proteins. *Nat Biotechnol* 2004;22:826–827.
- 22 Qing G, Ma LC, Khorchid A, Swapna GV, Mal TK, Takayama MM, Xia B, Phadtare S, Ke H, Acton T, Montelione GT, Ikura M, Inouye M: Cold-shock induced high-yield protein production in *Escherichia coli*. *Nat Biotechnol* 2004;22:877–882.
- 23 Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680–685.
- 24 Matsudaira P: Sequence from picomole quantities of proteins electrophoretically onto polyvinylidene difluoride membranes. *J Biol Chem* 1987;262:10035–10038.
- 25 Jeong KY, Lee J, Lee IY, Ree HI, Hong CS, Yong TS: Analysis of amino acid sequence variations and immunoglobulin E-binding epitopes of German cockroach tropomyosin. *Clin Diagn Lab Immunol* 2004;11:874–878.