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Analytical Methods

Development of sandwich ELISA for detection and quantification of invertebrate major allergen tropomyosin by a monoclonal antibody

Hong Zhang [a], Ying Lu [b], Hideki Ushio [a,c,*], Kazuo Shiomi [c]

[a] Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 1138657, Japan
[b] Department of College of Food and Technology, Shanghai Ocean University, Shanghai 201306, PR China
[c] Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Minato-ku, Tokyo 1088477, Japan

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ABSTRACT

Muscle protein tropomyosins of invertebrates are major allergens responsible for wide spread allergic reactions against invertebrates such as shellfish and insects. In order to develop a sandwich enzyme-linked immunoadsorbent assay (ELISA) for detection and quantification of the invertebrate pan-allergen tropomyosin, a specific monoclonal antibody (MAb), CE7B2, was produced. We have successfully established a sandwich ELISA for measuring invertebrate tropomyosin concentrations in food and food materials. The sandwich ELISA system using the MAb CE7B2 is a useful tool to detect and quantify levels of tropomyosin in food. The method is also helpful to detect mite and cockroach tropomyosins, the important indoor allergens.

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1. Introduction

Because of the high nutritive value, seafood including shellfish is an important food resource in the world. Shellfish such as crustaceans and mollusks are then important and common causes of food allergy (Daul & Morgan, 1993; Lopata, O'Hehir, & Lehrer, 2010). On the other hand, indoor allergies against dust mite and cockroach are now recognized as major clinical concerns (Arruda, 2005). Food and indoor allergies are commonly synonymous with type-I allergy that is mediated by immunoglobulin E (IgE) antibodies bound to mast cell. Following the reaction of allergen with specific IgE, the mast cell degranulates and releases histamine, leukotrienes and other mediators, causing hypersensitive reactions, such as urticaria, angioedema, asthma, rhinitis, vomiting, diarrhea and shock in severe cases (Daul & Morgan, 1993).

The major heat-stable allergen in shrimp was first described in Hoffman, Day, and Miller (1981) and later identified as tropomyosin (Shanti, Martin, Nagpal, Metcalfe, & Rao, 1993). Studies on food allergies demonstrated that tropomyosin is an invertebrate pan-allergen and shows cross-reactivities among the given invertebrate species (Reese, Ayuso, & Lehrer, 1999).

Detection kits for shellfish tropomyosin are commercially available in Japan (Seiki et al., 2007; Shibahara et al., 2007). However, tropomyosin is relatively labile in enzymatic processing (Hoffman et al., 1981), and the tropomyosin fragmented peptides containing IgE epitope sequences remain easily in processed food. Those detection kits without IgE epitope specificities might fail to detect allergenic peptides in processed foods, such as fermented products and fish sauce. The previous studies demonstrated that an amino acid sequence around the C terminal is shared among shellfish tropomyosins and that the sequence is strongly recognized by sera of human individuals sensitive to shellfish (Emoto, Ishizaki, & Shiomi, 2009). In the present study, a MAb CE7B2 specific to the sequence was used as a capture antibody in a sandwich ELISA system to detect invertebrate tropomyosin.

Immunological reaction-based techniques have been described to identify and quantify allergens in food including enzyme-linked immunosorbent assay (ELISA) (Jeoung et al., 1997). Even though some monoclonal antibodies (MAbs) had been obtained against invertebrate tropomyosin (Barletta et al., 2005; Lu, Ohshima, Ushio, Hamada, & Shiomi, 2007), it is necessary to develop an MAb recognizing the tropomyosin IgE epitopes shared by invertebrate species for rapid and highly sensitive detection. Although polyclonal antibodies (PAbs) are often used for the ELISA because of their low cost, PAbs have some weak points; PAb is finite; each lot of antibody, even though raised against the same antigen, shows different antigen recognition ability, which would reduce the reliability of the assay. MAbs possess a high level of selectivity

* Corresponding author at: Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 1138657, Japan. Tel.: +81 3 5841 7520; fax: +81 3 5841 8166.
E-mail address: aushio@mail.ecc.u-tokyo.ac.jp (H. Ushio).

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Table 1
Tropomyosin materials in this study.

Phylum	Class	Family	Species	Common name
Arthropoda	Malacostraca	Penaeidae	*Marsupenaeus japonicus*	Kuruma prawn
			Pandalus borealis	Pink shrimp
		Lithodidae	*Paralithodes camtschaticus*	King crab
		Atelecyclidae	*Erimacrus isenbeckii*	Horsehair crab
		Oregoniidae	*Chionoecetes japonicus*	Red snow crab
		Portunidae	*Portunus trituberculatus*	Swimming crab
	Arachnida	Pyroglyphidae	*Dermatophagoides pterouyssinus*	Dust mite
	Insecta	Blattidae	*Periplaneta fluiginosa*	American cockroach
Mollusca	Bivalvia	Veneridae	*Raditapes philippinarum*	Short-neck clam
		Mactridae	*Pseudocardium sachalinense*	Sakhalin surf clam
		Arcidae	*Anadara broughtonii*	Bloody cockle
		Pectinidae	*Patinopecten yessoensis*	Yezo scallop
	Gastropoda	Haliotidae	*Haliotis discus*	Disk abalone
		Buccinidae	*Neptunea polycostata*	Whelk
	Cephalopoda	Ommastrephidae	*Todarodes pacificus*	Japanese flying squid
		Octopodidae	*Octopus vulgaris*	Common octopus
Echinodermata	Holothuroidae	Holothuriidae	*Stichopus japonicus*	Sea cucumber
Chordata	Ascidiaca	Pyuridae	*Halocynthia roretzi*	Ascidian
	Actinopterygii	Sparidae	*Pagrus major*	Red sea bream
	Amphibia	Ranidae	*Rana catesbeiana*	American bullfrog
	Aves	Phasianidae	*Gallus gallus domesticus*	Chicken
	Mammalia	Bovidae	*Bos primigenius*	Cattle

Table 2
Coefficients of variation for intra- and inter-assay variations ($n = 3$).

Kuruma prawn (ng/ml)	Intra-assay (% CV)	Inter-assay (% CV)
0.75	5.1	4.2
12	1.5	1.2
96	2.2	1.8
192	3.4	2.8
600	3.4	2.8
Japanese flying aquid (ng/ml)		
1.28	0.8	0.6
41	3.9	3.2
164	2.4	1.9
328	1.4	1.2
512	3.1	2.2

CVs were defined as the standard deviation divided by the mean and multiplied by 100.

for a single epitope and can be produced in unlimited amounts. We then developed a sandwich ELISA using MAb in order to provide a promising tool for the specific detection and quantification of invertebrate tropomyosins and presumably allergenic fragmented peptides in daily foods and daily life.

2. Materials and methods

2.1. Materials

The tropomyosin materials used in this study are listed in Table 1. Fresh specimens of kuruma prawn (*Marsupenaeus japonicus*), pink shrimp (*Pandalus borealis*), king crab (*Paralithodes camtschaticus*), swimming crab (*Portunus trituberculatus*), red snow crab (*Chionoecetes japonicus*), horsehair crab (*Erimacrus isenbeckii*), short-neck clam (*Raditapes philippinarum*), disk abalone (*Haliotis discus*), Sakhalin surf clam (*Pseudocardium sachalinense*), bloody cockle (*Anadara broughtonii*), Yezo scallop (*Patinopecten yessoensis*), whelk (*Neptunea polycostata*), Japanese flying squid (*Todarodes pacificus*), common octopus (*Octopus vulgaris*), sea cucumber (*Stichopus japonicus*), ascidian (*Halocynthia roretzi*), red sea bream (*Pagrus major*), American bullfrog (*Rana catesbeiana*), chicken (*Gallus gallus domesticus*) and cattle (*Bos primigenius*) were obtained from local markets. Dust mite (*Dermatophagoides pterouyssinus*) extract and American cockroach (*Periplaneta fluiginosa*) whole body powder were obtained from Bio Stir (Tokyo, Japan).

The processed food extracts are listed in Table 2. The extracts from instant noodle soup mix of seafood style, Chinese-inspired bean-starch vermicelli soup mix (containing shellfish in materials), and Sichuan-inspired bean-starch vermicelli soup mix (showing no shellfish ingredient in the label on the package but a product through the same production line as for shellfish-containing products), shrimp cracker, octopus cracker, shrimp powder, fish sauce, kimchi (showing no shellfish ingredient in the label on the package) and kimchi sauce were obtained from Japanese local supermarkets.

All animal experiments in this study were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of Tokyo University of Marine Science and Technology and were approved by the Animal Experiment Committee in Tokyo University of Marine Science and Technology.

2.2. Preparation of tropomyosin-rich fractions and processed food extracts

The tropomyosin-rich acetone powder was prepared from each sample according to the method of Greaser and Gergely (1971). The powder was dissolved in 20 volumes of 50 mM phosphate buffer (pH 7.2), and subjected to salting-out (30–55% saturation of ammonium sulfate) to obtain myofibrillar proteins. The resulting supernatant containing tropomyosin was kept at 100 °C for 30 min because tropomyosin is more stable against heat treatment compared with other myofibrillar proteins (Hoffman et al., 1981). After centrifugation, the resulting supernatant was desalted through a PD-10 column (GE Healthcare Japan, Tokyo, Japan) and stored at −80 °C until use.

The processed food materials were separately incubated in 5 volumes of 50 mM phosphate buffer (pH 7.2) overnight at 4 °C. After centrifugation at 15,000 g for 25 min, the supernatant was desalted by the PD-10 column and stored at −80 °C until use.

2.3. Synthetic peptide design

The C-terminal region peptide sequence, NH_2-SISDELDQTFA-ELC-COOH, was designed based on the sequence of IgE epitope

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