Food Chemistry 114 (2009) 634–641

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Tropomyosins in gastropods and bivalves: Identification as major allergens and amino acid sequence features

Ai Emoto, Shoichiro Ishizaki, Kazuo Shiomi *

Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Konan-4, Minato-ku, Tokyo 108-8477, Japan

ARTICLE INFO

Article history:
Received 14 July 2008
Received in revised form 23 August 2008
Accepted 30 September 2008

Keywords:
Allergen
Amino acid sequence
Bivalve
cDNA cloning
Gastropod
Mollusc
Tropomyosin

ABSTRACT

Tropomyosin appears to be a major cross-reactive allergen of crustaceans and molluscs. In this study, four species of gastropods (disc abalone, turban shell, whelk and Middendorf's buccinum) and seven species of bivalves (bloody cockle, Japanese oyster, Japanese cockle, surf clam, horse clam, razor clam and short-neck clam) were confirmed to be allergenic by ELISA and their major allergen identified as tropomyosin by immunoblotting. Inhibition immunoblotting data showed the cross-reactivity of gastropod and bivalve tropomyosins with one another and also with cephalopod and crustacean tropomyosins. Then, amino acid sequences of tropomyosins from 10 species except for Middendorf's buccinum were elucidated by cDNA cloning. The known amino acid sequence data including our results reveal that molluscan tropomyosins share low sequence identities (about 60%) with crustacean tropomyosins and that they are highly homologous with one another within the same group (same family or same order) but not among the groups.

1. Introduction

Food allergy mediated by immunoglobulin E (IgE) antibodies is a public concern in industrialised countries. Sensitised subjects with high levels of IgE to a specific food can develop hypersensitive reactions, such as urticaria, asthma, diarrhoea and even anaphylaxis, immediately after ingestion of the food. In coastal countries, shellfish including crustaceans and molluscs are obviously among the most common causes of food allergy. The major allergen of crustaceans is tropomyosin, a 35–38 kDa myofibrillar protein involved in the muscle contraction, as demonstrated with the following various species: shrimps (Daul, Slattery, Reese, & Lehrer, 1994; Leung et al., 1994; Motoyama, Suma, Ishizaki, Nagashima, & Shiomi, 2007; Shanti, Martin, Nagpal, Metcalfe, & Subba Rao, 1993), crayfishes (Leung et al., 1998a), lobsters (Leung et al., 1998a), crabs (Leung et al., 1998b; Motoyama et al., 2007), krills (Motoyama et al., in press; Nakano, Yoshimura, & Yamada, 2008), mantis shrimps (Motoyama et al., in press) and barnacles (Suma et al., 2007). Moreover, tropomyosin is considered to be a major allergen in molluscs primarily composed of cephalopods, gastropods and bivalves. Molecular evidence for this has been obtained with as many as nine species of cephalopods (cuttlefish, squid and octopus) (Ishikawa, Suzuki, Ishida, Nagashima, & Shiomi, 2001; Miyazawa et al., 1996; Motoyama, Ishizaki, Nagashima, & Shiomi, 2006). However, despite that gastropods and bivalves include a variety of groups, molecular studies have been limited to the following species: Japanese abalone *Haliotis diversicolor* (Chuo, Wong, & Leung, 2000), turban shell *Turbo cornutus* (Ishikawa, Ishida, Shimakura, Nagashima, & Shiomi, 1998a), green mussel *Perna viridis* (Chuo et al., 2000), noble scallop *Chlamys nobilis* (Chuo et al., 2000), Japanese oyster *Crassostrea gigas* (Ishikawa, Ishida, Shimakura, Nagashima, & Shiomi, 1998b; Ishikawa, Shimakura, Nagashima, & Shiomi, 1997) and constricted tagelus *Sinonovacula constricta* (Song, Li, Li, & Ran, in press).

IgE cross-reactivity is clinically and experimentally recognised among crustaceans, among molluscs and even between crustaceans and molluscs (Lehrer & McCants, 1987; Leung et al., 1996; Motoyama et al., 2006; Reese, Ayuso, & Lehrer,1999). Accumulated data on the primary structures of crustacean tropomyosins prove that they share extremely high sequence identities (mostly more than 90%) with one another (Motoyama et al., 2007), except for barnacle tropomyosin having considerably low identities (about 60%) with other crustacean tropomyosins (Suma et al., 2007). In accordance with this, the eight IgE epitopes proposed for brown shrimp *Penaeus aztecus* tropomyosin (Pen a 1) (Ayuso, Lehrer, & Reese, 2002b; Ayuso, Reese, Leong-Kee, Plante, & Lehrer, 2002a; Reese et al., 2005) are well conserved in other crustacean tropomyosins, being a molecular basis for the cross-reactivity among crustaceans. On the other hand, the cross-reactivity either among molluscs or between crustaceans and molluscs is not fully understood due to the shortage of information about the primary

* Corresponding author. Tel.: +81 3 5463 0601; fax: +81 3 5463 0669.
E-mail address: shiomi@kaiyodai.ac.jp (K. Shiomi).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.09.100

structures of molluscan tropomyosins, especially those of gastropod and bivalve tropomyosins. Amino acid sequences of gastropod and bivalve tropomyosins even including those with no evidence for allergenicity are known only for three species of abalones, three species of mussels, three species of scallops and one species of clam (refer to Table 2).

For a better understanding of the cross-reactivity either among molluscs or between crustaceans and molluscs, it is essential to elucidate amino acid sequences of allergenic tropomyosins from various species of gastropods and bivalves. In this study, therefore, four species of gastropods (disc abalone, turban shell, whelk and Middendorf's buccinum) and seven species of bivalves (bloody cockle, Japanese oyster, Japanese cockle, surf clam, horse clam, razor clam and short-neck clam), which are situated at various taxonomical positions (refer to Table 1), were selected as samples. We report here the identification of tropomyosins as the major allergens in these 11 species and the amino acid sequences of tropomyosins in 10 species (except for Middendorf's buccinum) determined by a cDNA cloning technique. The sequence features of molluscan tropomyosins are also discussed compared to those of crustacean tropomyosins.

2. Materials and methods

2.1. Animal samples

Live specimens of four species of gastropods (disc abalone *Haliotis discus discus*, turban shell *T. cornutus*, whelk *Neptunea polycostata* and Middendorf's buccinum *Buccinum middendorffi*), seven species of bivalves (bloody cockle *Scapharca broughtonii*, Japanese oyster *C. gigas*, Japanese cockle *Fulvia mutica*, surf clam *Pseudocardium sachalinensis*, horse clam *Tresus keenae*, razor clam *Solen strictus* and short-neck clam *Ruditapes philippinarum*) and American lobster *Homarus americanus* and fresh specimens of Japanese flying squid *Todarodes pacificus* were all purchased at the Tokyo Central Wholesale Market. The taxonomical positions of the gastropods and bivalves are summarised in Table 1. Columellar muscle was obtained from disc abalone, foot muscle from turban shell, whelk, Middendorf's buccinum, bloody cockle, Japanese cockle, surf clam and razor clam, adductor muscle from Japanese oyster, siphon (major edible muscle part) from horse clam, soft tissues from short-neck clam, abdominal muscle from American lobster and mantle muscle from Japanese flying squid. Muscle or soft tissue samples for extraction were stored at −20 °C until use and those for molecular cloning experiments were immediately immersed in liquid nitrogen and stored at −80 °C until use.

Table 1
Gastropods and bivalves used in this study.

Class	Order	Family	Species (common name)
Gastropoda	Vetigastropoda	Haliotidae	*Haliotis discus discus* (Japanese abalone)
		Turbinidae	*Turbo cornutus* (turban shell)
	Neogastropoda	Buccinidae	*Neptunea polycostata* (whelk)
			Buccinum middendorffi (Middendorf's buccinum)
Bivalvia	Arcoida	Arcidae	*Scapharca broughtonii* (bloody cockle)
	Ostreoida	Ostreidae	*Crassostrea gigas* (Japanese oyster)
	Veneroida	Cardiidae	*Fulvia mutica* (Japanese cockle)
		Mactridae	*Pseudocardium sachalinensis* (surf clam)
			Tresus keenae (horse clam)
		Solenidae	*Solen strictus* (razor clam)
		Veneridae	*Ruditapes philippinarum* (short-neck clam)

2.2. Preparation of heated extracts

Each muscle or soft tissue sample was homogenised with four volumes of 0.15 M NaCl in 0.01 M phosphate buffer (pH 7.0). In view of the fact that tropomyosin, a target protein in this study, is thermostable, the homogenate was then heated in a boiling water bath for 10 min. After centrifugation at 18,000g for 15 min, the supernatant obtained was used as a heated extract. Protein concentrations of the heated extracts were quantified by the method of Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin as a standard.

2.3. Purification of tropomyosin

American lobster, Japanese flying squid and turban shell tropomyosins were purified from the abdominal, mantle and foot muscles, respectively, as reported previously (Motoyama et al., 2007). In brief, an acetone powder of myofibrillar proteins prepared from the muscle was 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 to salting-out with ammonium sulfate (30–60% saturation), followed by isoelectrical precipitation (pH 4.6). Finally, small amounts of contaminants were removed by reverse-phase HPLC on a TSKgel ODS-120T column (0.46 × 25 cm; Tosoh, Tokyo, Japan). The homogeneity of the final preparation was supported by SDS–PAGE.

2.4. Human sera

Sera were donated from 10 crustacean-allergic patients (patients 1–10) with a history of hypersensitive reactions after ingestion of crustaceans. These patients had all been diagnosed to be allergic to crustaceans by the capsulated hydrophilic carrier polymer-radioallergosorbent test (CAP-RAST) performed at hospitals; the determined CAP-RAST classes were 3–6 against shrimp and/or crab. Patients 5, 6, 8 and 9 had been additionally shown to have CAP-RAST classes of 3–6 against squid and octopus. In this study, sera from eight healthy volunteers without adverse reactions after ingestion of any foods were used as controls.

2.5. Fluorescence ELISA

Reactivity of the patient sera to each heated extract was evaluated by fluorescence ELISA as described elsewhere (Hamada et al., 2004). In brief, a polystyrene microtiter plate with 96 wells (Type H (black); Sumitomo Bakelite, Tokyo, Japan) was coated with 50 μl of the heated extract (diluted 1:2000) and reacted successively with patient or control serum (diluted 1:500) and β-galactosidase-conjugated goat anti-human IgE antibody (0.25 μg/ml; American Qualex, San Clement, CA, USA). Enzyme reaction was carried out using substrate solution (0.1 mg/ml 4-methylumbelliferyl-β-D-galactoside) and stopped by the addition of 100 mM glycine-NaOH buffer (pH 10.3). Fluorescence intensity was measured on a SPECTRAmax GEMINI XS (Molecular Devices, Sunnyvale, CA, USA) with excitation at 367 nm and emission at 453 nm. All ELISAs were performed in triplicate and the data obtained were expressed in mean ± SD.

2.6. SDS–AGE

SDS–PAGE was performed on a PhastSystem apparatus (GE-Healthcare, Piscataway, NJ, USA) as described in the manufacturer's manual. Ready-made gels (PhastGel Gradient 8-25) and ready-made buffer strips (PhastGel SDS Buffer Strips) were purchased from GE-Healthcare. Each sample was dissolved in 62.5 mM phosphate buffer (pH 7.5) containing 2.5% SDS and 5% dithiothreitol, heated at 100 °C for 10 min and subjected to