Food Chemistry 141 (2013) 4031–4039

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Impact of heat processing on the detection of the major shellfish allergen tropomyosin in crustaceans and molluscs using specific monoclonal antibodies

Sandip D. Kamath [a], Anas M. Abdel Rahman [b,c], Toshikazu Komoda [d], Andreas L. Lopata [a,*]

[a] School of Pharmacy and Molecular Science, Centre for Biodiscovery and Molecular Development of Therapeutics, James Cook University, Townsville, Queensland, Australia
[b] Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada
[c] Department of Chemistry, Memorial University of Newfoundland, St. John's, NL, Canada
[d] School of Food, Agricultural and Environmental Sciences, Miyagi University, Sendai, Miyagi, Japan

ARTICLE INFO

Article history:
Received 10 January 2013
Received in revised form 2 June 2013
Accepted 24 June 2013
Available online 3 July 2013

Keywords:
Tropomyosin
Monoclonal antibodies
Seafood
Allergen heat-treatment
IgE epitope recognition
Crustacean
Mollusc

ABSTRACT

The major heat-stable shellfish allergen, tropomyosin, demonstrates immunological cross-reactivity, making specific differentiation of crustaceans and molluscs for food labelling very difficult. The aim of this study was to evaluate the application of allergen-specific monoclonal antibodies in differential detection of shellfish-derived tropomyosin in 11 crustacean and 7 mollusc species, and to study the impact of heating on its detection. Cross-reactive tropomyosin was detected in all crustacean species, with partial detection in molluscs: mussels, scallops and snails but none in oyster, octopus and squid. Furthermore, we have demonstrated that heating of shellfish has a profound effect on tropomyosin detection. This was evident by the enhanced recognition of multiple tropomyosin variants in the analysed shellfish species. Specific monoclonal antibodies, targetting the N-terminal region of tropomyosin, must therefore be developed to differentiate tropomyosins in crustaceans and molluscs. This can help in correct food labelling practices and thus protection of consumers.

1. Introduction

Seafood plays an important role in human nutrition and health. The growing international trade in seafood species and products has added to the popularity and frequency of consumption of a variety of seafood products across many countries. This increased production and consumption of seafood has been accompanied by more frequent reporting of allergic health problems among consumers. Allergic reactions are manifested by gastrointestinal and dermatological symptoms, as well as respiratory and anaphylactic reactions (Lopata & Lehrer, 2009; Lopata, O'Hehir, & Lehrer, 2010). The appearance of allergic symptoms results not only from ingestion of seafood; it can also be triggered by inhaling cooking vapours and handling shellfish (Jeebhay & Lopata, 2012; Jeebhay, Robins, Lehrer, & Lopata, 2001; Lopata & Jeebhay, 2013). Importantly, patients with shellfish allergy, similarly to those with peanut allergy, mostly remain clinically reactive throughout their lives and are at increased risk of wheezing illness and hyper-reactive airways at school age (Lopata, O'Hehir, & Lehrer, 2010).

Food allergy to shellfish is on an increase, affecting approximately 2% of the general population. Several commercially important shellfish are used as food additives or supplements in a number of consumer food products (e.g. oyster sauce, krill oil). Accidental exposure to food products, cross-contaminated with shellfish allergens during processing, can occur and is an important consumer health concern.

The three most important seafood groupings causing allergic reactions include fish, crustacea and mollusc. The latter two phyla of crustaceans and molluscs are generally referred to as 'shellfish' in the context of seafood consumption. The allergic response in sensitised consumers is mediated by serum IgE antibodies directed to specific allergens, such as the major allergen tropomyosin, an abundant shellfish muscle protein (Albrecht et al., 2008). The presence of this very same allergenic protein in processed food, even at very low concentrations, can cause severe reactions in sensitised consumers. Therefore the labelling of food products containing crustaceans has already become mandatory in many countries, including the USA, Europe and Japan. Recently the European Union adapted guidelines to include molluscs as a separate food allergen, based on the limited cross-reactivity to crustacean allergens (Opinion of the Scientific Panel on Dietetic products, 2006).

* Corresponding author. Address: Molecular Immunology Group, School of Pharmacy and Molecular Science, Centre for Biodiscovery and Molecular Development of Therapeutics, Building 21, Molecular Sciences, James Cook Drive, Douglas Campus, James Cook University, Townsville, Queensland 4811, Australia. Tel.: +61 (07) 47814563; fax: +61 (07) 47816078.
E-mail address: andreas.lopata@jcu.edu.au (A.L. Lopata).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.06.105

Commercially available shellfish allergen detection kits usually make use of polyclonal rabbit antibodies. However their ability to differentiate between the major allergens from crustaceans and molluscs is often not defined and, in the case of such polyvalent rabbit antibodies, it is very difficult to achieve.

The aims of this study were to evaluate the use of allergen-specific monoclonal antibodies for the detection of shellfish-derived tropomyosin in a comprehensive range of crustacean and mollusc species and to analyse the impact of heat-processing on antibody recognition for improved allergen detection in processed food.

2. Materials and methods

2.1. Shellfish samples

Fresh or frozen specimens of 11 different crustacean and 7 mollusc species were acquired from local markets and distributors across Melbourne, Australia, as listed in Table 1. The specimens were transported to the laboratory on ice and frozen at −20 °C prior to further use.

2.2. Preparation of protein extracts

For the preparation of raw protein extract, the outer shell of the specimen was removed and the edible meat cut into small pieces. The abdominal or tail muscles were used from prawns, crabs and lobster specimens. For the bivalves, the shell was split open and the inner muscle parts used for extraction. About 50 g of the muscle mass was homogenised in 150 ml of phosphate buffered saline (PBS) for 10 min, using an Ultra turrax blender (IKA, Staufen, Germany). This slurry was then agitated for 3 h at 4 °C, followed by centrifugation at 14,000 rpm for 15 min. The supernatant was clarified through a glass fibre filter, followed by filtration through a 0.45 μm membrane filter (Millipore, Billerica, MA, USA) and stored at −80 °C prior to further use.

For the generation of heated protein extracts, a more natural way of heat treatment was utilised, instead of just heating the raw extract, to mimic the way consumers are usually exposed to food allergens. The complete shellfish specimen, in its outer shell, was heated in liquid (PBS) at 100 °C for 20 min. The outer shell was removed after cooling and the proteins from these muscle tissues extracted using the same method as described above.

2.3. Protein quantification

The total protein content of each prepared extract was determined using the Quick Start Bradford Assay kit (BioRad, USA), following the manufacturer's instructions. Bovine serum albumin (BSA) was used as the protein standard.

2.4. SDS–PAGE analysis

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed to visualise the total protein repertoire in the prepared extracts, as described previously (Abdel Rahman, Kamath, Gagne, Lopata, & Helleur, 2013; Abdel Rahman et al., 2010). Twelve microgram of protein extract were briefly heated in Laemmli buffer with dithiothreitol and loaded onto a 12% bisacrylamide gel. Electrophoretic separation was performed at 170 V until the tracker dye reached the base, using a Mini-Protean Tetra Cell electrophoresis system (BioRad, Hercules, CA, USA). The separated proteins were visualised by staining with Coomassie brilliant blue R250 (BioRad, Hercules, CA, USA).

2.5. Immunoblotting

2.5.1. Immunoblotting with monoclonal anti-tropomyosin antibody

Four microgram of the crustacean protein extract were resolved by SDS–PAGE, as detailed above. The separated proteins were transferred to an activated polyvinylidene fluoride (PVDF) membrane, using the Semi-dry TransBlot Apparatus (BioRad, Hercules, CA, USA). After blocking with 5% (w/v) skim milk powder (SMP) in PBS-T, the membrane was subsequently incubated with monoclonal anti-insect tropomyosin antibody, mac-141 (Abcam, Cambridge, MA, USA) diluted 1:6000 in 1% SMP, PBS-T and rabbit anti-mouse IgG antibody conjugated with HRP (Sigma, St. Louis, MO, USA) diluted 1:50,000. After washing three times with PBS-T, the membrane was visualised using the enhanced chemiluminescent technique, as reported previously (Abdel Rahman, Kamath, Lopata, & Helleur, 2010; Abdel Rahman, Kamath, Lopata, Robinson, & Helleur, 2011). Briefly, the blots were incubated with chemiluminescent substrate (Sigma, St. Louis, MO, USA) and exposed to photographic film (GE Healthcare Biosciences, USA) to visualise the antibody-binding protein bands.

Table 1
Common and scientific names of the eleven crustacean and seven mollusc species analysed in this study. The theoretical molecular weight and GenBank accession numbers of characterised tropomyosins are listed for each species if available.

No.	Shellfish species				Theoretical MW (kDa)	Accession numbers (GenBank)
			Common name	Scientific name		
1	Crustaceans	Prawn	Black tiger prawn	*Penaeus monodon*	32.8	HM486525
2			King prawn	*Melicertus latisulcatus*	32.6	JX171685
3			Vannamei prawn	*Litopenaeus vannamei*	32.8	EU410072
4			Banana prawn	*Fenneropenaeus merguiensis*	32.8	GU369817
5			Green tiger prawn	*Penaeus semisulcatus*	–	–
6		Crab	Blueswimmer crab	*Portunus pelagicus*	32.8	JX874982
7			Sand crab	*Ovalipes australiensis*	-	–
8			Snow crab	*Chionocetes opilio*	32.6	BAF47267
9		Lobster	Slipper lobster	*Thenus orientalis*	32.0	KC291443
10			Rock lobster	*Jasus edwardsii*	32.9	KC291442
11			Yabby	*Cherax destructor*	32.0	KC291443
12	Molluscs	Bivalve	Green mussel	Perna viridis	32.7	AAG08988
13			Blue mussel	*Mytilus edulis*	32.7	U40035
14			Scallop	*Pecten fumatus*	–	–
15			Oyster	*Crassostrea gigas*	33.0	BAH10152
16		Gastropod	Sea snail	*Turbo cornutus*	32.7	AB444940
17		Cephalopod	octopus	*Octopus vulgaris*	32.8	BAE54433
18			Calamari (squid)	*Sepioteuthis lessoniana*	32.6	AB218914