



Local bivalve allergens

A Malaysian perspective

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Abstract

Background Apart from the nutritional benefits, bivalves are also considered to be among a group of allergenic foods. Symptoms of allergy to bivalves are largely similar to those of allergic reactions reported for other shellfish. The identification of allergenic proteins in a particular species is an important step for the development of more accurate allergy tests and for the definition of more effective management of patients. Currently, studies regarding the allergens in bivalve species are lacking.

Materials and methods This minireview is an overview of local scientific articles on identification of bivalve allergens by allergenomics methods including sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional gel electrophoresis (2-DE), immunoblotting and mass-spectrometry analysis.

Results Tropomyosin has been the only well recognized allergen in a wide range of bivalve species. In addition to tropomyosin, our local study resulted in discovery of three novel bivalve major allergens; arginine kinase (40 kDa), actin (42 kDa) and troponin C (18 kDa).

Conclusion This minireview will provide a better understanding of each allergen derived from local bivalves which subsequently will help in the un-

derstanding of the specific immunoglobulin E (IgE)-mediated immune response.

Keywords Allergen · Bivalve · Tropomyosin · Arginine kinase · Actin · Troponin C · Mollusca

Abbreviations

2-DE	Two dimensional-gel electrophoresis
cDNA	Complementary deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide-gel electrophoresis

Introduction

Bivalves belong to the invertebrate Phylum *Mollusca*, which also includes gastropods and cephalopods. The name “bivalve” refers to the two-part shell that characterizes these shellfish species. Some well-known bivalves include cockles, clams, oysters, mussels and scallops [1, 2].

Bivalves are valued as food sources and recommended in several dietary regimes for their high protein content, low fat or cholesterol profile, the presence of good lipids, significant amounts of omega-3-fatty acids, dietary essential amino acids, vitamin B₁₂ and several important minerals such as zinc, iron and copper [3–5]. In Malaysia, total production of bivalves was approximately 20,311 tons and it was estimated that the retail value of bivalve's production amounted to RM 253,000 million [6]. Amongst the bivalve species, five species of local bivalves commonly consumed were Malaysian cockle *Anadara granosa*, carpet clam *Paphia textile*, tropical oyster *Crassostrea belcheri*, Asiatic hard clam *Meretrix meretrix* and Asian green mussel *Perna viridis*.

Apart from the nutritional benefits, bivalves are also considered to be among a group of allergenic foods

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[7, 8]. The prevalence of allergy to bivalves varies throughout the world. It is likely to parallel consumption patterns being more frequent in locales where consumption is high [9]. Immunoglobulin E (IgE)-mediated bivalve allergy has been described in several countries including France [10], Spain [11], South Africa [12], Japan [13, 14], Canada [15], Italy [16, 17], Germany [18], Sweden, Denmark, Estonia Lithuania and Russia [19]. Our earlier study among 131 atopic patients in the Allergy Clinic, Hospital Kuala Lumpur demonstrated that patients in Malaysia also suffering from allergic responses to bivalves. Amongst the bivalve extracts tested, Malaysian cockle elicited the highest frequency of positive skin test reactivity in 18% ($n=24$) of the subjects. Carpet clam was the second most common bivalve to elicit a positive reaction in the skin prick test (SPT), 15%, followed by Asiatic hard clam, 8%, tropical oyster, 6% and Asian green mussel, 5% [20]. Allergies to bivalves have also been documented in work places that handle bivalves. These have been found to occur primarily through inhalation of aerosols generated during cutting, scrubbing or cleaning, cooking or boiling and drying [21]. Reactions can also occur through the skin as a result of direct contact to the bivalves [22].

Symptoms of bivalve's allergy are more or less similar to that of other shellfish allergies. Common symptoms include mild oral allergy (itching of the lips, mouth and throat and swelling of the lips, tongue, throat and palate), cutaneous reactions (urticaria, eczema), respiratory symptoms (asthma, rhinitis), gastrointestinal symptoms (diarrhoea, vomiting) and systemic reactions (anaphylactic shock) [9].

At present, allergies to bivalves are diagnosed similarly to other shellfish allergies. The diagnosis relies upon a careful evaluation of history, the presence of appropriate clinical signs and confirmed with in vivo or in vitro tests to demonstrate the presence of allergen-specific IgE [1, 9]. In Malaysia, skin prick test (SPT) is commonly used by allergy specialists to detect shellfish-specific IgE including bivalves. Serum immunoassays to determine specific IgE antibodies provide another modality to evaluate IgE-mediated bivalve allergy. To date, the most commonly used system to determine allergen-specific IgE is the ImmunoCAP test (Thermo Fisher Scientific, Uppsala, Sweden). However, the accuracy of this test is restricted to the panel of commercially available species. Currently, the bivalve species available for testing is limited to several species such as clam (*Ruditapes spp*), oyster (*Ostrea edulis*), blue mussel (*Mytilus edulis*) and scallop (*Pecten spp*); species which are not commonly consumed in Malaysia.

In our country, the only prophylactic approach to prevent allergic reactions to bivalve is a specific avoidance diet. With bivalve allergies, individuals are advised to avoid the ingestion of one or more species of bivalve. Often, patients with bivalve allergies are advised to avoid all molluscan shellfish species or all

shellfish (both molluscan and crustacean) or even all seafood. In addition to strict avoidance, the symptoms of bivalve allergy may be mitigated by pharmacological treatment. Symptoms are usually treated with antihistamines, anticholinergic agents and corticosteroids or epinephrine in case of anaphylaxis.

By definition, allergy results from adverse immunological responses to proteins or antigens, which are referred to as allergens [1, 23]. Allergens are often characterized by having the following traits: the ability to sensitize a genetically predisposed individual by triggering the production of IgE antibodies, the ability to bind those particular IgE antibodies and the ability to cause an allergic reaction following IgE binding [23].

In the case of bivalve allergy, allergy testing is mostly performed using crude allergen extracts. A major problem using crude allergen extracts for testing is that it is difficult and often impossible to precisely identify the disease-eliciting allergen. IgE results may be due to cross-reactive allergens instead of the specific allergens to a given biological source [24]. Therefore, identification of allergens is of the utmost importance because it has the capability in resolving whether sensitization of patients towards a given allergen source was genuine or due to cross-reactive allergens [25]. Identification of allergens is also helpful in predicting the severity of allergic reactions. Allergens stable to heat and digestion causing reactions also to cooked foods and often associated with systemic and more severe allergic reactions. In contrast, allergens sensitive to heat and digestion seldom associated with clinical symptoms but may cause local reactions. Allergens can be defined as being either major or minor. "Major allergens" are proteins that elicit IgE binding in the majority, more than 50% of patients; while those which elicit allergic reactions only in a minor fraction, less than 50% of patients allergic to the specific source, are regarded as "minor allergens" [1].

Currently, studies regarding the allergens in bivalve species are lacking. To date, tropomyosin has been the only well-recognized major allergen in a wide range of bivalve species (Table 1). Emoto et al. [26] isolated 37 kDa allergens from the bloody cockle *Scapharca broughtonii* and the Japanese cockle *Fulvia mutica* and identified them as tropomyosins. Emoto et al. [26] further reported the 37 kDa major allergens in the surf clam *Pseudocardium sachalinensis*, razor clam *Solen strictus*, horse clam *Tresus keenae*, short-neck clam *Ruditapes philippinarum* and Japanese oyster *Crassostrea gigas* as tropomyosins. In this study, seven species of bivalves (bloody cockle, Japanese cockle, surf clam, razor clam, horse clam, short-neck clam and Japanese oyster) were confirmed to be allergenic by enzyme-linked immunosorbent assay (ELISA) and their major allergens were identified as tropomyosin by immunoblotting. Then, amino acid sequences of tropomyosins were elucidated by complementary

Table 1 Summary of bivalve allergens with known biochemical identities

Source of allergen	Nomenclature	MW (kDa)	Biochemical identity	Accession number	Reference
Bivalves					
<i>Chlamys (Mimachlamys) nobilis</i> (scallop)	Chl n 1	38	Tropomyosin	AF216520	Chu et al. [28]
<i>Crassostrea gigas</i> (Pacific oyster)	Cra g 1	37	Tropomyosin	AB444943	Leung and Chu [27]
<i>Fulvia mutica</i> (Japanese cockle)	–	37	Tropomyosin	AB444944	Emoto et al. [26]
<i>Perna viridis</i> (green mussel)	Per v 1	38	Tropomyosin	AF216519	Chu et al. [28]
<i>Pseudocardium sachalinensis</i> (surf clam)	–	37	Tropomyosin	AB444945	Emoto et al. [26]
<i>Ruditapes (Venerupis) philippinarum</i> (short-neck clam)	–	37	Tropomyosin	–	Emoto et al. [26]
<i>Scapharca broughtonii</i> (bloody cockle)	–	37	Tropomyosin	AB444942	Emoto et al. [26]
<i>Solen strictus</i> (razor clam)	–	37	Tropomyosin	AB444947	Emoto et al. [26]
<i>Tresus keenae</i> (horse clam)	–	37	Tropomyosin	AB444946	Emoto et al. [26]

deoxyribonucleic acid (cDNA) cloning. The amino acid sequence data revealed that tropomyosins share sequence identities (about 60%) with crustacean tropomyosins [26].

The major allergen in the Pacific oyster *Crassostrea gigas* has also been identified as tropomyosin [27]. In this study, Leung and Chu [27] cloned, identified and determined the primary structure of a major IgE-reactive mollusc allergen in the oyster at the DNA and protein level. *Cra g* 1.03 was identified as the first oyster allergen at the molecular level. *Cra g* 1.03 has an open reading frame of 233 amino acids and demonstrates marked similarity in amino acid composition and peptide sequence with mollusc and crustacean tropomyosins.

Tropomyosins have also been identified in the green mussel *Perna viridis* and the scallop *Chlamys nobilis* [28]. In this study, the cDNAs encoding tropomyosins of the mussel and scallop were amplified by reverse transcriptase polymerase chain reaction and thereafter cloned in plasmid vectors for expression. Immunoblot analysis showed that recombinant proteins of mussel and scallop tropomyosins were reactive to serum IgE antibodies from subjects allergic to shellfish but not to non-allergic controls. Nucleotide and amino acid sequences of these mussel and scallop tropomyosins are similar (mostly >55%) to those of crustacean allergens identified as tropomyosins.

Over the years, invertebrate's tropomyosin is a well-known allergenic protein. Tropomyosin is a 34 to 38 kDa heat-stable protein that plays a functional role in contractile activities in muscle cells [29]. Allergenic tropomyosins have been identified in various crustaceans and molluscs [26, 30–33]. Tropomyosin is a highly conserved protein among various invertebrate species and demonstrates a high amino acid sequence identity. Because of this, IgE antibodies raised against tropomyosin from a certain species may bind to and trigger an allergic reaction upon exposure to tropomyosin from a different source [34]. IgE cross-reactivity of tropomyosin can be seen clinically and experimentally amongst crustaceans, amongst mol-

luscs, between crustaceans and molluscs and between crustaceans and terrestrial arthropods, such as cockroaches and mites [29, 30]. However, in contrast to invertebrate tropomyosins, vertebrate tropomyosins appear to be non-allergenic, possibly because of their greater susceptibility to breakdown by digestive enzymes compared to invertebrate tropomyosins [34]. Using bioinformatics approaches to compare the sequences of tropomyosins from various species, Goodman et al. [35] determined that tropomyosins from vertebrate species such as rabbit, pig, chicken and human share 53–57% amino acid sequence identity to the known shrimp tropomyosin allergen, Met e 1. This comparison also helps to explain why vertebrate tropomyosins are not allergenic and do not cross-react with IgE antibodies specific to invertebrate tropomyosins.

Aside from tropomyosin, our local study has added a noticeable number of allergens to the meagre list of bivalve allergens reported so far, namely: arginine kinase, actin and troponin C. The 40 kDa major allergen found in Malaysian cockle *Anadara granosa* was identified as arginine kinase, while the 42 kDa major allergen in carpet clam *Paphia textile* was identical to actin. The 18 kDa major allergen in Asian green mussel *Perna viridis* was identified as troponin C.

Tropomyosin

It is known that tropomyosin has been the only well-recognized allergen in a wide range of bivalve species [26–28]. Similarly, our study has also identified tropomyosin as one of the major allergen in all five species of local bivalves studied. The major allergens of 36 kDa found in Malaysian cockle *Anadara granosa*, Asiatic hard clam *Meretrix meretrix* and Asian green mussel *Perna viridis* and the 37 kDa in carpet clam *Paphia textile* and tropical oyster *Crassostrea belcheri* were identified as tropomyosin [36–39]. Tropomyosin with a molecular weight about 34 to 38 kDa, is a group of highly conserved protein which plays a central role in muscle contraction [29]. Tropomyosin is heat resistant and retains its IgE-binding ability even after

prolonged heating [29]. Our study has also confirmed and strengthened earlier reports on the heat stability of tropomyosin protein. Earlier in our study, immunoblots of the cooked bivalve extracts showed that it still retained the 36–37 kDa major allergen found in the raw extracts as it bound IgE from patients' sera [36–39]. The ability of tropomyosin to withstand heat-treatment and most known type of food processing techniques can be attributed to its exceptionally stable alpha helical coiled-coil secondary structure [29].

Moreover, it is also known that tropomyosin is more allergenic in cooked form, as the cooking process alters the epitopes of the allergen. Previous experiments using shrimp tropomyosin displayed an increase in allergen potency with the progression of the Maillard reaction [40]. Carnés et al. [41] also discovered an increase in tropomyosin reactivity following boiling using in vivo SPT where boiled shrimp tropomyosin elicited larger skin wheals than untreated tropomyosin samples. Our study is in accordance with other reports which suggested that tropomyosin is more allergenic in cooked form, as majority of the sera had markedly increased IgE-binding intensity to the tropomyosin protein (36–37 kDa) in the cooked bivalve extracts [36–39] suggesting that some new epitopes might have been exposed for IgE-binding [40]. However, clinical studies are not conducted in our study to support this finding.

Arginine kinase

Aside from tropomyosin, arginine kinase has been identified as the second major allergen of Malaysian cockle *Anadara granosa* [36]. Arginine kinase is a phosphagen kinase that plays a vital role in energy metabolism in invertebrates [31]. Arginine kinase has been identified as one of the significant allergen in crustaceans and some invertebrates such as shrimps or prawns, crayfish, crab, moth, house dust mite, cockroach and spider [31, 32, 42]. However, arginine kinase is obviously less IgE reactive and also less thermostable than tropomyosin, suggesting that arginine kinase contributes less to the adverse reactions induced after ingestion of cockle, especially cooked cockle, than tropomyosin [36].

Actin

In addition to tropomyosin, actin has been identified for the first time as the second major allergen of carpet clam *Paphia textile*. It is a heat-sensitive protein with a molecular weight of 42 kDa [37]. Thus far, only Abdel Rahman et al. [42] has identified α -actin as an allergenic protein in the snow crab *Chionoecetes opilio*. Actin is an important contractile protein in eukaryotic cells. It is one of the two major components involved in the contraction of muscle cells. In non-muscle cells, it is the major part of the cytoskeleton

involved in many processes such as cell motility, endocytosis, exocytosis, phagocytosis, organelle movement, material transportation and signal transduction [43].

Troponin C

Our study is the first to report troponin C as the second major allergen of Asian green mussel *Perna viridis*. Troponin C with a molecular weight about 18 kDa was identified as a heat-stable allergen because this protein was recognised by sera in immunoblotting of the thermal-treated Asian green mussel [39]. Troponin C is a subunit of troponin which is involved in calcium-dependent muscle contraction in skeletal and cardiac muscle [44]. Prior to our study, troponin C has already been identified to be one of the allergens in crustacean and some invertebrates such as shrimps or prawns, cockroach and mite [44, 45].

Conclusions

In addition to tropomyosin, our local study had added a noticeable number of allergens to the meagre list of bivalve allergens reported so far, namely: arginine kinase, actin and troponin C. These allergens seem particularly important to improve diagnosis and management of local bivalve allergic patients. However, identification of these allergens was only detected by allergenomics methods including sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional gel electrophoresis (2-DE), immunoblotting and mass-spectrometry analysis.

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Author's contributions Z.H.M. Yadzir: interpretation of results and analysis, writing the paper. R. Misnan, F. Bakhtiar, S. Samsudin, N. Abdullah: interpretation of results and analysis, critical revision of the paper.

Conflict of interest Z.H.M. Yadzir, R. Misnan, F. Bakhtiar, S. Samsudin and N. Abdullah declare that they have no competing interests.

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