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Shellfish allergens: tropomyosin and beyond

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Abstract

IgE-mediated shellfish allergy constitutes an important cause of food-related adverse reactions. Shellfish are classified into molluscs and crustaceans, the latter belonging to the class of arthropoda. Among crustaceans, shrimps are the most predominant cause of allergic reactions and thus more extensively studied. Several major and minor allergens have been identified and cloned. Amongst them invertebrate tropomyosin, arginine kinase, myosin light chain, sarcoplasmic calcium-binding protein and hemocyanin are the most relevant. This review summarizes our current knowledge about these allergens.

Key words: allergy, allergens, cross-reactivity, crustaceans, specific IgE; molluscs, shrimp

Abbreviations:

GDPH; Glyceraldehyde Phosphate DeHydrogenase

MLC; myosin light chain

SCP; Sarcoplasmic Calcium-binding Protein

SERCA; Smooth Endoplasmic Reticulum Ca⁺⁺ ATPase

sIgE; specific Immunoglobulin E

TpC; Troponin-C

Introduction

IgE-mediated shellfish allergy constitutes an important and increasing health issue in both children and adults (1, 2). During the last 2 decades significant progress in biochemistry and molecular biology enabled the characterization, cloning and recombinant production of various shellfish allergen components and epitope emulating peptides that might become available for quantification of specific IgE (sIgE) antibodies, namely molecular diagnosis. This review intends to summarize our current knowledge about shellfish allergens and their cross-reactivity as this might be the key to optimize diagnosis (3, 4).

Allergens

Table 1 summarises the most relevant shellfish allergens that have currently been characterized. Tropomyosin is considered to be the major allergen in shellfish allergy. Actually, already in the early eighties Hoffman *et al* (5) identified a heat-stable IgE-binding allergen in shrimps, that was later identified as tropomyosin in brown shrimp (*Penaeus aztecus*) reacting with 28/34 (82%) of shrimp-sensitive individuals (6). Moreover, tropomyosin has been identified as a pan-allergen of many invertebrate species including other crustaceans (lobster, crab), molluscs (mussels, oysters, scallops, octopus, squids, snails, abalones, whelk, clams, razor shell), cockroaches and mites (7-18). Tropomyosins are present in both muscle and non-muscle cells. In striated muscle they mediate the interaction of troponin-actin complex to regulate contraction. Note that tropomyosins from crustaceans share a high homology (up to 98%), whereas the amino acid sequence identity between crustacean and mollusc tropomyosin is lower, about 65%. Vertebrate

tropomyosins, although they share about 55% of sequence homology with invertebrate tropomyosins, seem to be non-allergenic (12). At present, different IgE-binding B cell and putative T cell epitopes (some of them found clinically relevant) have been described in tropomyosin (3, 19-25). Children were once reported to recognize a greater epitope repertoire than adults and thus suggested that shrimp sensitization could decrease over age (22). However, more recent studies with challenged patients could not reproduce this observation with the same epitope mapping (3, 24). About one decade ago, a recombinant tropomyosin from *Penaeus aztecus*, rPen a 1, became commercially available for molecular diagnostic testing, with improved results as a diagnostic tool in comparison with the whole shrimp extract (26, 27).

In addition to tropomyosin, several other allergenic components have been identified in shellfish. In 2003 Yu *et al* identified a novel allergen in *Penaeus monodon* (black tiger shrimp) (28), designated as Pen m 2, with arginine kinase activity. Similar to tropomyosin, arginine kinase is highly abundant in invertebrate muscle and hitherto, it has also been described in various other shellfish species as well as other invertebrates such as mites, cockroaches, crickets, silk worms and the Indian meal moth (29-40). Unlike tropomyosins, they display unstable physicochemical properties and do not resist thermal and acid-base treatment (41, 42).

A third allergen is myosin light chain (MLC) that was first described by Ayuso *et al* (43) in *Litopenaeus vannamei* (Pacific white shrimp) and designated as Lit v 3. Subsequently it was also described in various taxonomically distant and related invertebrates, such as other shrimp species and cockroaches (22, 44, 45). Like tropomyosin and arginine kinase, shrimp MLC display a significant amino acid sequence homology with homologues in other

arthropods such as Bla g 8 from *Blatella germanica* (German cockroach) (43). MLC molecules are highly resistant to heat, acid–alkali and digestion, and retain weakly IgE-binding activity when their secondary structure is altered (46).

A forth shellfish allergen is the heat resistant sarcoplasmic calcium-binding protein (SCP), an EF-hand-type protein. It constitutes an allergen in various crustaceans such as shrimp and crayfish (22, 38, 40, 44, 45, 47-49). Like other allergenic components, SCPs from taxonomically related species demonstrate a significant amino acid sequence homology that varies between 80-98% for crustaceans, whereas homology between crustacean and molluscan SCPs is only 15-21% (50). Similar to shrimp tropomyosin, various epitopes have been identified for shrimp MLC, AK and SCP and its potential as diagnostic tools has been assessed (22, 24).

Hemocyanin, isolated from hemolymph is another heat stable shrimp allergen (36, 38, 51-53) and cross-reacts with its homologue in house dust mite (38) and probably also snail (54). In one of these studies, the authors elegantly demonstrated that sensitization to hemocyanin can lead to a selective allergy to *Macrobrachium rosenbergii* (giant fresh water shrimp) in patients tolerating a DBPCFC with *Penaeus monodon* (black tiger shrimp) (51). The explanation for these selective allergies should probably be sought in the low amino acid sequence homology (only 19-27%) between both shrimp species that respectively belong to the *Caridae* and *Penaidae* family.

Troponin-C (TpC) was first described as an allergen in *Crangon crangon* (Cra c 6, North Sea shrimp) (45), recognized by 9/31 (29%) of the patients and later in *Pandalus borealis* (northern shrimp), recognized by 33% of the patients (4). In a recent study by Pascal *et al* (3)

sensitization to TpC was found in 17.2% of 58 shrimp allergic patients. TpC has also been identified in cockroach (55) and storage mites (*Tyrophagus putrescentiae*) (56).

Other potential allergens identified in shellfish are paramyosins (identified in many mollusc species (57) and *Anisakis simplex* (58)), triose phosphate isomerases such as Cra a 8 (44, 45), myosin heavy chain (52), α - and β -actin (18, 37, 40), smooth endoplasmic reticulum Ca^{++} ATPase (SERCA) (37), glyceraldehyde phosphate dehydrogenase (GDPH), titin (44) and ubiquitin (40), however a deeper characterization is still required.

As indicated in the Table 1, several allergenic components such as tropomyosin, MLC and SCP are resistant to heat. Moreover, boiling might even enhance their allergenicity (16, 59), e.g. by creating neo-epitopes during Maillard reactions (60). In the study by Jirapongsananuruk *et al* (61), the proportion of positive challenges to raw shrimp was lower than to cooked shrimp. For a review on the effect of various physical and chemical treatments on the allergenicity of various shellfish tropomyosins the reader is referred elsewhere (2).

Cross-reactivity

Cross-reactivity between shellfish species is a common phenomenon and involves several components that are currently extending beyond the major allergen tropomyosin. As a matter of fact, cross-reactivity between shellfish species might involve almost all allergenic components that have been identified so far. However, this paragraph focuses on tropomyosin as most of our knowledge gathered today relates to this component. As mentioned above and displayed in Table 2, amino acid homology between the tropomyosins

of phylogenetically related crustaceans attains up to 98% (62, 63). For molluscan shellfish, a comparable intra-class amino acid similarity exists for tropomyosins of gastropods (85-91%) (64, 65), cephalopods (91-100%) (66) and bivalves (70-100%). The homology between the different molluscan classes varies from 68-100%. In contrast, amino acid homology between crustacean and molluscan tropomyosin is lower, 56-68% (66). For example, sequence homology between Tropomyosin 1 from the cephalopod *Todarodes pacificus* and Penodomyosin 1 from *Penaeus orientalis* (Chinese white shrimp) is 62% (8). There is 81% of amino acid homology of tropomyosin between shrimp and mites, and 82% between shrimp and cockroach. Sequence homology between tropomyosin from shrimp and the nematode *Anisakis simplex* (Anisakis 3) is 75% (67) and between shrimp and fish (e.g. tilapia (Oreochromis 4, *Oreochromis mossambicus*) is about 55% (68)). Obviously, this high molecular homology of shellfish tropomyosins translates to significant degrees of IgE cross-reactivity between various shellfish species (8, 9, 62, 63, 65, 69, 70) or between shellfish and other invertebrates (7, 12-14, 21, 40, 67, 71-74). For example, Leung *et al* demonstrated that sera from shrimp allergic patients contain IgE antibodies against molluscs, grasshopper, cockroach and fruit fly but not to chicken or mouse (69). Furthermore, from these studies it appears that particularly house dust mite and cockroach tropomyosins have been demonstrated to account for the presence of specific IgE to shrimp, even in unexposed subjects such as Orthodox Jews who abstain from shellfish according to kosher law (75). The opposite, with the occurrence of mite and cockroach allergy as a result of primary sensitization to crustaceans has also been suggested (21). Besides that, species-specific allergies have been demonstrated (61, 76, 77), as demonstrated by Jirapongsananuruk *et al* (61), in which the idea of shellfish allergy as a pan-allergy was rejected by demonstrating specific allergies to *Penaeus monodon* and *Macrobrachium rosenbergii*.

With this in mind, the clinical recommendation that all patients with shellfish allergy should strictly avoid all crustaceans, molluscs and edible insects may not be absolutely accurate. Larger studies with challenge-documented patients focusing on the understanding of clinical cross-reactivity are absolutely mandatory to better understand and properly manage shellfish allergy. It is also a general misbelieve that shellfish allergens cross-react with fish allergens. As a matter of facts, the major fish allergen is parvalbumin that cross-reacts with other parvalbumins (e.g. in frog) (78). Tropomyosin is not a major allergen in fish. Actually the particular finding of a high prevalence of sIgE antibodies to tropomyosin found in fish allergic patients published by Liu *et al* (68) probably results from the fact that the majority of patients had inflammatory bowel disease, and these patients probably synthesise antibodies against human tropomyosin isoform 5 that has a C-terminal peptide virtually identical to Ore m 4, the tropomyosin of tilapia.

Summary

As witnessed from the literature, through the last 2 decades significant advances have been made in the characterization, cloning and recombinant production of different major and minor protein components and epitope-emulating peptides from various shellfish species. Further deciphering of the complex IgE reactivity profiles of shellfish allergy and the availability of these components and peptides might shift paradigms in diagnostic and treatment approaches.

Table 1.

Allergenic components in shellfish (adapted and updated from (44) and (2))					
COMPONENT	MW (KDa)	ALLERGEN NATURE AND FUNCTION	HEAT RESISTANCE	EXAMPLES (NOT EXHAUSTIVE)	ROUTE OF EXPOSURE
Tropomyosin	34-39	Muscle contraction	Yes	Pen a 1, Lit v 1, Tod p 1, Der p 10, Der f 10, Bla g 7, Per a 7, Hom a 1, Cra c 1, Cha f 1, Mac ro 1,	Ingestion Inhalation
Arginine kinase	40-45	Energy for muscle contraction	No	Pen m 2, Lit v 2, Cra c 2, Art fr 2, Der p 20, Bla g 9, Per a 9	Ingestion Inhalation
Myosin light chain	17-20	Muscle contraction	Yes	Lit v 3, Cra c 5	Ingestion
Sarcoplasmic calcium-binding protein	20-25	Regulation muscle contraction	Yes	Lit v 4, Pen m 4, Cra c 4	Ingestion
Hemocyanin	+ 75	Copper containing protein – oxygen carrier	Yes	Bla g 3, Per a 3	
Troponin-C	17.7	Muscle contraction / relaxation	Yes	Cra c 6, Bla g 6	
Triose phosphate isomerase	26-29	Glycolysis	No	Cra c 8	Ingestion Inhalation
Paramyosin	100	Muscle contraction			
Titin	70	Passive muscle elasticity	Yes		Ingestion
Fructose 1,6 biphosphate aldolase	39-43	Glycolysis	No		Ingestion Inhalation
Myosin heavy	225	Muscle contraction			

chain					
α -actin	31-42	Muscle contraction			
SERCA	113	Enzyme			
GADPH	37	Enzyme			
Ubiquitin	8.5	Enzyme	No		
Pen a: <i>Penaeus aztecus</i> (brown shrimp), Lit v: <i>Litopenaeus vannamei</i> (Pacific white shrimp), Pen m: <i>Penaeus monodon</i> (black tiger shrimp), Art fr: <i>Artemia franciscana</i> (San Francisco brine shrimp, Tod d : <i>Todarodes pacificus</i> (Japanse common squid), Der p: <i>Dermatophagoides pteronyssinus</i> (house dust mite), Der f: <i>D. farinae</i> (flour mite), Bla g: <i>Blatella germanica</i> (German cockroach), Per a: <i>Periplaneta americana</i> (American cockroach), Hom a: <i>Homarus americanus</i> (American lobster), Cra c: Crangon crangon (North Sea shrimp), Cha f: <i>Charybdis feriatus</i> (crucifix crab), Mac ro: <i>Macrobrachium rosenbergii</i> (giant fresh water shrimp).For allergen components see www.allergome.org and www.allergen.org MW= Molecular Weight.					

Table 2.

Allergen (TM)	Pen a 1	Pen m 1	Mac r 1	Hom a 1	Der p 10	Bla g 7	Ani s 3
Brown shrimp (<i>Penaeus aztecus</i> , Pen a 1)	100	100	98	93	80	82	73
Black tiger shrimp (<i>Penaeus monodon</i> , Pen m 1)	100	100	98	93	80	82	73
Giant fresh water shrimp (<i>Macrobrachium rosenbergii</i> , Mac ro 1)	98	98	100	93	80	83	76
American lobster (<i>Homarus americanus</i> , Hom a 1)	93	93	92	100	93	83	75
House dust mite (<i>Dermatophagoides pteronyssinus</i> , Der p 10)	80	80	80	83	100	73	N/A
German cockroach (<i>Blatella germanica</i> , Bla g 7)	82	82	83	83	73	100	69
<i>Anisakis simplex</i> (Ani s 3)	73	73	76	75	N/A	69	100

Allergen (AK)	Pen m 2	Der p 20	Per a 9
Black tiger shrimp (<i>Penaeus monodon</i> , Pen m2)	100	79	83
House dust mite (<i>Dermatophagoides pteronyssinus</i> , Der p 20)	79	100	75
American cockroach (<i>Periplaneta americana</i> , Per a 9)	83	75	100

Allergen (MLC)	Pen m 3	Hom a 3	Bla g 8
Black tiger shrimp (<i>Penaeus monodon</i> , Pen m 3)	100	N/A	62
American lobster (<i>Homarus americanus</i> , Hom a 3)	N/A	100	N/A
German cockroach (<i>Blatella germanica</i> , Bla g 8)	62	N/A	100

Allergen (Tp C)	Pen m 6	Hom a 6	Bla g 6
Black tiger shrimp (<i>Penaeus monodon</i> , Pen m 6)	100	77	70
American lobster (<i>Homarus americanus</i> , Hom a 6)	77	100	63
German cockroach (<i>Blatella germanica</i> , Bla g 6)	70	63	100

Allergen (HC)	Mac r hemocyanin	Bla g 3
Giant fresh water shrimp (<i>Macrobrachium rosenbergii</i> , <i>Mac ro Hemocyanin</i>)	100	28
German cockroach (<i>Blatella germanica</i> , Bla g 3)	28	100

Sequence homology between Tropomyosins (TM), Arginine Kinase (AK), Myosin Light Chain (MLC), Troponin C (TpC) and Hemocyanin (HC) according to <http://www.uniprot.org/> and <http://www.ncbi.nlm.nih.gov/genbank/>. N/A: Not available

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We attest to the fact that all authors listed on the title page have contributed significantly to the work, have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission. We have no conflict of interest to declare.

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