

Allergenic Tropomyosins and Their Cross-Reactivities

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Abstract: The ingestion or inhalation of some proteins may lead to adverse immune reactions. Allergens may trigger allergic reactions in genetically predisposed individuals when they are absorbed through the skin or make contact with mucous membranes. An allergic disease often deteriorates the quality of life and may sometimes be life-threatening due to anaphylactic shock.

A number of allergens have been characterized from various multicellular organisms to date. It is thought to be reasonable to pay a special attention to the substance which is highly cross-reactive and which causes adverse responses in the molecules that are not sensitized but similar to the sensitized allergen.

Tropomyosin has been described as an important food allergen in shrimp, lobster, crab, oysters, squid, and other invertebrates. Allergic reactions to shellfish and mollusks are often cross-reactive, which may be explained by the highly conserved amino acid sequences of tropomyosins among invertebrates, but vertebrate tropomyosins are not known to be allergenic. Several tropomyosins from domestic arthropods have been reported to be allergenic. Recently, it was suggested that an infection of helminthic parasites might lead to sensitization to tropomyosin and elicit allergic reactions to other invertebrates.

Much effort has been made to characterize these allergenic tropomyosins from various sources. We will discuss the physicochemical characteristics and the potential application of tropomyosin for the diagnosis and therapeutics of allergic disorders.

Keywords: Allergen, cross-reactivity, tropomyosin.

INTRODUCTION

Food allergies to shellfish such as shrimp, lobster, crayfish, and crab, as well as mollusks such as squid, octopus, snails, clams, mussels, oysters and scallops, are relatively common and may be life-threatening [1, 2]. A severe anaphylactic reaction was reported to be induced after a good-night kiss from a friend who had eaten shrimp prior to the kiss [3].

Seven allergens were detected by crossed immunoelectrophoresis using sera from six subjects who tested positive to shrimp extract with both the skin test and the radioallergen sorbent test (RAST) [4]. However, only two classes of allergens have been cloned and characterized from crustacea to date. The first was tropomyosin, which was identified from a heat-stable fraction as an abundant and highly soluble allergen, demonstrated strong IgE-binding activity and was found to dominate the allergenicity of the shrimp extract [5]. The application of techniques from molecular biology to the study of allergens has facilitated the cloning and allergenic characterization of tropomyosins from other invertebrates [6]. The second of these allergens was arginine kinase, which was identified by proteomic analysis of *Penaeus monodon*

extract, and its cDNA was cloned by rapid amplification of cDNA ends (RACE)-PCR and named Pen m 2 [7]. The studies on their allergenic cross-reactivity indicated that common epitopes are present in crustacea, mite, and insect arginine kinases. However, only about 27% (5 of 18) of shrimp allergy sera showed a significant IgE reactivity with Pen m 2. Arginine kinase has also been described as an allergen in the moth, *Plodia interpunctella* (Plo i 1), and it was suggested as a new class of invertebrate pan allergens [8]. Arginine kinase reacted with serum IgE from 25% (13 of the 52) of moth-allergic patients.

Evidence has attributed the cross-reactivity between insects, mites, nematodes, and crustacea to tropomyosin [9-13]. Cross-reactivity between different organisms is believed to occur due to identical or similar IgE-binding epitopes [14].

The identification and characterization of allergens, including cross-reactive allergens that elicit allergic disorders may provide the fundamental information needed for the diagnosis and therapeutic approach for these allergic diseases [15,16].

STRUCTURE AND FUNCTION OF TROPOMYOSIN

Tropomyosin and the troponin C, I, and T subunits regulate the contraction of skeletal muscle. The cytosolic Ca^{2+} concentration influences the position of these proteins on the actin thin filaments, which in turn controls the interactions

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between the actin and the myosin. Tropomyosin is a ropelike molecule and is approximately 40 nm in length. Two parallel alpha-helical tropomyosin molecules are wound around each other to form a coiled-coil structure [17]. Coiled-coil structures have been characterized by the occurrence of a heptad repeat (abcdefg) (Fig. 1). Generally, hydrophobic amino acids occupy the 'a' and 'd' positions, while polar or ionic amino acids occur at other positions. Hydrophobic residues in the positions 'a' and 'd' are involved in the interaction between the two alpha-helices. The interactions between acidic residues in position 'e' and basic residues in position 'g' also contribute to the stabilization of the coiled-coil. The other amino acid residues at positions 'b', 'c', and 'f' occupy the outer positions and interact with other proteins such as actin and troponin [18,19]. The tropomyosin molecule also forms a continuous chain along each actin thin filament by head-to-tail polymerization. Each tropomyosin molecule binds to seven actin monomers in a thin filament. The position of contact between tropomyosin and an actin filament is through troponin I, troponin T, and the calcium-binding subunit, troponin C subunits (Fig. 2) [20].

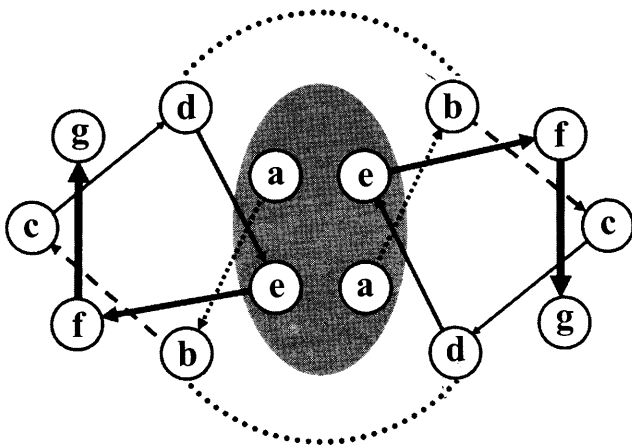


Figure 1. Helical wheel representation of one heptad repeat of the individual α -helices, illustrating how the side chains of residues 'a' and 'd' pack together in coiled-coils.

Tropomyosin molecules have been isolated from both muscle and non-muscle cells. Various isoforms of tropomyosin have been purified from bovine thyroid [21], porcine kidney [22], rabbit lung macrophage [23], human erythrocyte [24,25], porcine platelet [26], and chicken embryo fibroblast [27]. Tropomyosin was described to be involved in the localization of mRNA in a *Drosophila* embryo [28,29]. Tropomyosins in non-muscle cells are believed to function for the mechanical support of the cytoplasmic membrane [30] and in the transportation of other molecules. Cells from different embryonic lineages express different isoforms and the syntheses of these isoforms are developmentally regulated [31]. There are at least 18 different isoforms known to be generated by alternative RNA splicing in mammalian cells.

ALLERGENIC TROPOMYOSINS

Leung et al. (1996) were able to detect IgE reactivity in six different species of arthropods and ten mollusks using shrimp-sensitive sera [11]. The organisms investigated in the study were shrimp (*Metapenaeus ensis*), spiny lobster (*Pa-*

nulirus homarus), mud crab (*Scylla serrata*), grasshopper (*Mecopoda elongata*), cockroach (*Periplaneta americana*), fruit fly (*Drosophila melanogaster*), abalone (*Haliotis diversicolor*), whelk (*Hemifusus ternatana*), mussel (*Perna viridis*), pen shell (*Pinna atropurpurea*), scallop (unidentified), oyster (*Crassostrea gigas*), clam (*Lutraria philipinarum*), cuttlefish (*Sepia madokai*), squid (*Loligo edulis*), and octopus (*Octopus luteus*). DeWitt et al. (2004) identified cross-reactive allergens using recombinant shrimp tropomyosin (rPen a 1) from other invertebrates, such as shrimp (*Pandalus borealis*), lobster (*Homarus gammarus*), crab (*Cancer pagurus*), blue mussel (*Mytilus edulis*), octopus (*Octopus vulgaris*), house dust mite (*Dermatophagoides pteronyssinus*), and German cockroach (*Blattella germanica*) [32]. However, IgE antibodies directed against invertebrate tropomyosins did not recognize the vertebrate tropomyosins. Ayuso et al. (1999) also reported that muscle protein tropomyosin is not an important vertebrate meat allergen after intensive examination of vertebrate meats including beef, lamb, pork, venison, chicken, and turkey [33]. Tropomyosin has been identified as the major common allergen from crustaceans and mollusks. Furthermore, it was suggested as an invertebrate 'pan allergen' based on the high degree of sequence identity and cross-reactivity among invertebrates [6]. We will discuss here the tropomyosins according to their taxonomic categories. Allergenic tropomyosins of which cDNA sequences are available are summarized in Table 1.

TROPOMYOSINS AS FOOD ALLERGENS

SHRIMP TROPOMYOSINS

Tropomyosin was recognized and characterized as an important allergen found in shrimp. The most frequent sign of shrimp-induced allergies is generalized itching (90%). The other common adverse reactions are the development of hives (61%), swelling of the lips and tongue (52%), pulmonary symptoms (42%), gastrointestinal symptoms (35%), and anaphylactic shock (13%) [34]. The major heat-stable allergen was isolated from Indian shrimp, *Penaeus indicus*, and designated as Sa-II [35]. Further investigation revealed that the allergen was tropomyosin [5] and was subsequently renamed according to allergen nomenclature as Pen i 1 [36]. Other research groups have also reported that the muscle protein tropomyosin was identified as the major shrimp allergen, such as in brown shrimp (*Penaeus aztecus*, Pen a 1) [37,38], common shrimp (*Crangon crangon*) [9], and greasyback shrimp (*Metapenaeus ensis*, Met e 1) [39]. Indirect evidence is also available from other species of shrimp, such as Taiwan shrimp (*Parapenaeus fissurus*, Par f 1) [40], oriental shrimp (*Penaeus orientalis*) [41], northern red shrimp (*Pandalus borealis*) [32,42], black tiger shrimp (*Penaeus monodon*) [7, 43], and white shrimp (*Penaeus setiferous*) [44]. Tropomyosin is recognized by 80-90% of shrimp-allergic sera and inhibits most (85%) IgE reactivity to shrimp whole body extract, which indicates that tropomyosin is responsible for most of the allergenic activity of shrimp [37].

LOBSTER TROPOMYOSINS

Using serum IgE from patients who have showed anaphylactic reactions to shrimp, a cross-reactive allergen was identified from Indian spiny lobster (*Panulirus homarus*) [11]. Subsequent studies have identified and characterized

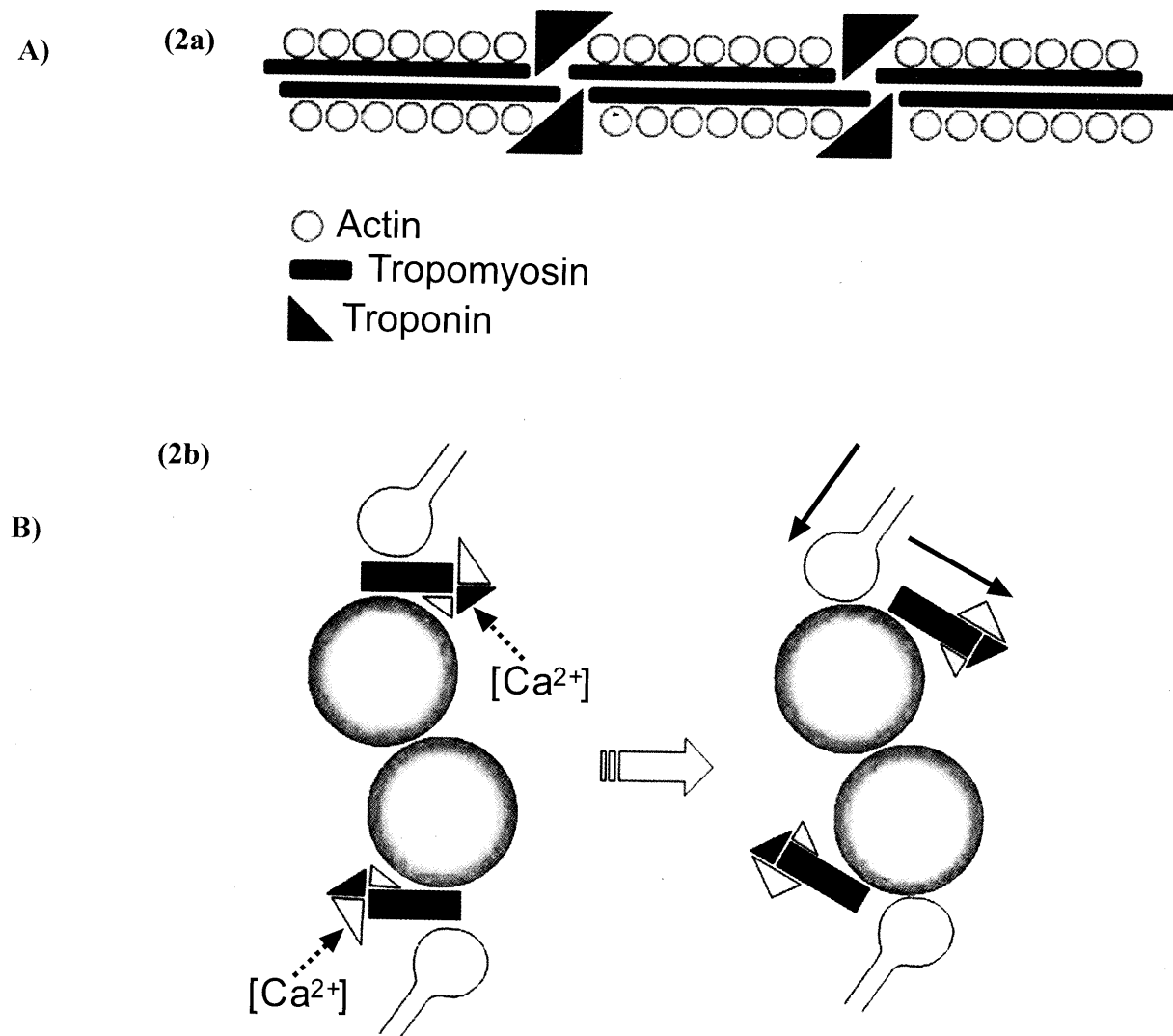


Figure 2. Schematic representation of tropomyosin in muscle. (A) Arrangement of tropomyosin and troponin in the actin filament. (B) Under resting conditions, the troponin complex, consisting of subunits C, T, and I, bonds in such a way with the actin and tropomyosin which causes the tropomyosin to interfere sterically with the attachment of the myosin head to the myosin binding sites of the actin (left). An increase in sarcoplasmic Ca^{2+} and its binding to troponin C causes a change in subunit affinities and a consequent shift of the tropomyosin away from the myosin-binding sites, which allows for the interaction of actin and myosin, resulting in a muscle contraction (right).

tropomyosin as the major allergen from other edible lobsters. American lobster (*Homarus americanus*) tropomyosin, isolated from striated muscle [45], was recognized by IgE from patients with crustacean allergies and was given the name Hom a 1 [46]. At the same time, the spiny lobster (*Panulirus stimpsoni*) tropomyosin (Pan s 1) was cloned and characterized by the screening of the cDNA library with sera of crustacean allergic subjects [46]. In addition, a cross-reactive allergen with recombinant shrimp tropomyosin (Pen a 1) from European lobster (*Homarus gammarus*) was identified [32]. However, detailed studies have not yet been performed. Lobster tropomyosins have been described as a major allergen to shellfish allergic subjects.

CRAB TROPOMYOSINS

Initially, a 38-kDa protein, which was possibly tropomyosin, was identified as a highly cross-reactive allergen from mud crab (*Scylla serrata*) [11]. In another study, a cDNA encoding for a 264-amino acid protein (Cha f 1) was isolated

from the common crab (*Charybdis feriatus*) from the cDNA library [47]. The resulting recombinant protein showed IgE reactivity to most of the crab-allergic individuals and the IgE reactivity to the crab extract could be inhibited by the absorption of sera with Cha f 1. It also showed a high cross-reactivity with shrimp tropomyosin, Met e 1. A cross-reactive allergen to shrimp tropomyosin (Pen a 1) from rock crab (*Cancer pagurus*) was also identified [32]. More allergenic tropomyosins could be identified if we were to investigate other edible crabs.

MOLLUSK TROPOMYOSINS

Leung *et al.* has identified cross-reactive allergens from several species of shellfish including crustacea and mollusca [11]. Further examination has been performed in some species. However, much remains to be investigated. Here, we have summarized and grouped the organisms into the following groups for convenience: gastropods, bivalvia, and cephalopods.

Table 1. Organisms from which Allergic Tropomyosins have been Cloned

Source	Allergen	Accession number	Ref number
Phylum Arthropoda			
Class Crustacea			
shrimps			
brown shrimp (<i>Penaeus aztecus</i>)	Pen a 1	DQ151457	37
Indian shrimp (<i>Penaeus indicus</i>)	Pen i 1		5
greasyback shrimp (<i>Metapanaeus ensis</i>)	Met e 1	U08008	39
black tiger shrimp (<i>Penaeus monodon</i>)	Pen m 1	AI253938 AI253947	7, 43
lobsters			
spiny lobster (<i>Panulirus stimpsoni</i>)	Pan a 1	AF030063	47
American lobster (<i>Homerus americanus</i>)	Hom a 1	AF034953-4	45
crabs			
common crab (<i>Charybdis feriatus</i>)	Cha f 1	AF061783	46
Class Arachnida			
mites			
house dust mite (<i>Dermatophagoides farinae</i>)	Der f 10	D17682	85
house dust mite (<i>D. pteronyssinus</i>)	Der p 10	AF016278	81
storage mite (<i>Blomia tropicalis</i>)	Blo t 10	AJ250096	86
storage mite (<i>Lepidoglyphus destructor</i>)	Lep d 10	AJ250096	87
storage mite (<i>Tyrophagus putrescentiae</i>)			
Class Insecta			
silverfish (<i>Lepisma saccharina</i>)	Lep s 1	AJ309202	107
cockroaches			
American cockroach (<i>Periplaneta americana</i>)	Per a 7	AF106961, Y14854	42, 79
German cockroach (<i>Blattella germanica</i>)	Bla g 7	AF260897	89
dusky-brown cockroach (<i>Periplaneta fuliginosa</i>)	Per f 7	AF454866	91
flies			
fruitfly (<i>Drosophila melanogaster</i>)			
nonbiting midge (<i>Chironomus ktiensis</i>)	Chi k 10	AJ012184	100
Phylum Mollusca			
Class Gastropoda			
abalones			
California red abalone (<i>Haliotis rufescens</i>)		X75218	
small abalone (<i>Haliotis diversicolor</i>)		AF216518	11, 48
snail (<i>Biomphalaria glabrata</i>)		M85199, M97554	
brown garden snail (<i>Helix aspersa</i>)	Hel a 1	Y14855	54
Turban (<i>Turbo cornutus</i>)	Tur c 1		55

(Table 1) contd...

Source	Allergen	Accession number	Ref number
whelk			
Class Bivalvia			
mussels			
blue mussel (<i>Mytilus edulis</i>)		U40035	32
green mussel (<i>Perna viridis</i>)		AF216519	48
pen shell			
scallops			
scallop (<i>Chlamys nobilis</i>)		AF216520	48
scallop (<i>Chlamys nipponensis</i>)		AB021681	
scallop (<i>Patinopecten yessoensis</i>)		AB050985	57
oyster			
Pacific oyster (<i>Crassostrea gigas</i>)	Cra g 1	AF239173	114
Clam			
Class Cephalopoda			
cuttlefish			
squid			
pacific squid (<i>Todarodes pacificus</i>)	Tod p 1		41
octopus			
Phylum Nematoda			
Anisakis (<i>Anisakis simplex</i>)	Ani s 3	Y19221	71
roundworm (<i>Ascaris lumbricoides</i>)			64

TROPOMYOSINS FROM GASTROPODS

A cross-reactive allergen from abalone, *Haliotis diversicolor*, was identified in 1996 [11] and its cDNA was cloned in 2000 [48]. Lopata *et al.* (1997) have detected a 38-kDa protein which is stable even after boiling and drying, which was possibly tropomyosin, as an important abalone (*Haliotis midae*) allergen. They also identified a 49-kDa protein (Hal m 1) [49]. In the same study, a similar protein of 38 kDa with IgE reactivity was also detected from abalone, *Haliotis rubra*, by Western blot analysis. A monoclonal antibody (AE9F9) prepared against Japanese abalone, *Haliotis discus*, was found to react to the crustacean tropomyosins, but did not react to the mollusks other than the abalone [50]. Monoclonal antibodies raised against the polymorphic regions might be useful for shellfish allergen detection and for the epitope analysis of allergenic tropomyosins.

A tropomyosin from whelk, *Pulgilina terntana*, was shown to be cross-reactive with other allergenic tropomyosins [11]. In a study on the Korean population, 24 patients were found to have high serum specific IgE levels to common whelk (*Buccinum undatum*) [51]. Their serum IgE reactivity was significantly inhibited by abalone, indicating cross-reactivity. Three IgE-binding components (40, 71, 82

kDa) were described by IgE immunoblotting. No detailed molecular or biochemical data is available on the whelk allergens as of yet.

It was reported that immunotherapy for house dust mite allergy may be accompanied by the induction of novel IgE antibody responses against the green garden snail (*Helix aspersa*) and shrimp [52]. Among the 17 serum samples investigated, two patients demonstrated oral allergy syndrome after eating shrimp, indicating that an adverse reaction against invertebrate tropomyosin may occur. It was also described that snail consumption may cause severe asthmatic symptoms in house dust mite allergic patients from Italy [53]. However, results of immunoblot analyses of these patients indicate that tropomyosin seems to play only a minor role as a cross-reactive allergen.

More recently, a cDNA sequence encoding tropomyosin from the brown garden snail (*Helix aspersa*) was cloned (Hel a 1) and its recombinant protein was produced [54]. It reacted with 18% of the sera from snail-allergic subjects and was found to represent a minor allergen in snail extract. However, inhibition experiments showed that the recombinant protein of the brown garden snail had various degrees of cross-reactivity with other invertebrate tropomyosins.

The major allergen from the horned turban (*Turbo cornutus*) was purified and named Tur c 1 [55]. The analyses of the amino acid composition and of the partial amino acid sequence identified Tuc c 1 to be a tropomyosin.

TROPOMYOSINS FROM BIVALVIA

Tropomyosin from the tropical green mussel (*Perna viridis*) was also identified as an important cross-reactive allergen [11], and its cDNA was cloned by reverse transcriptase (RT)-PCR [48]. A study using shrimp tropomyosin (Pen a 1) has also identified a cross-reactive allergen from the blue mussel (*Mytilus edulis*) [32]. However, no detailed data on the allergenicity of mussel tropomyosins are available as of yet. More extensive examination of mussel allergens is necessary because it is one of the most commonly consumed foods.

A cross-reactive allergen from clam (*Lutraria philippinarum*) which is possibly tropomyosin has also been described [11]. However, uncommon episodes from Spain were described after the intake of razor clams (*Ensis macha*) [56]. IgE-immunoblotting experiments revealed three major proteins; however, none of these were cross-reactive with tropomyosin from crustaceans, mollusks, house dust mites or cockroaches. The clinical relevance of clam tropomyosins remains to be evaluated. A cross-reactive allergen was also detected from pen shell (*Pinna atropurpurea*) [11], however, further studies have not been carried out on pen shell allergens.

The cDNA clones coding for tropomyosins from scallop (*Chlamys nobilis*), abalone (*Haliotis diversicolor*) and mussel (*Perna viridis*) were obtained by RT-PCR [48]. Absorption studies using their recombinant proteins demonstrated that tropomyosin is the major allergen among these edible mollusks. Tropomyosins were isolated from the Ezo giant scallop (*Mizuhopecten yessoensis*) and the Yesso scallop (*Patiopecten yessoensis*), and the change in allergenicity was investigated by studying the structural change caused by a reduction in sugars (the Maillard reaction) [57]. The results strongly suggested that the manner of cooking these scallops might influence the allergenicity of their food allergens.

By screening the cDNA library of the Pacific oyster (*Crassostrea gigas*) a cDNA encoding tropomyosin was isolated using sera from 15 oyster allergic patients, and was designated as Cra g 1.03 [58]. IgE reactivity to oyster extract was completely removed by the absorption of oyster allergic sera with its recombinant protein. Most of the IgE reactivity to Cra g 1.03 was also removed by the absorption of Met e 1, Pan s 1, and Cha f 1.

TROPOMYOSINS FROM CEPHALOPODS

Cross-reactive allergens from squid (*Loligo edulis*), cuttlefish (*Sepia madokai*), and octopus (*Octopus luteus*) have been identified [11]. A major allergen from the Pacific flying squid (*Todaroes pacificus*) was purified by column chromatography. Amino acid sequence analysis showed a marked homology with tropomyosin and the allergen was named Tod p 1 [41]. A cross-reactive allergen with shrimp allergen Pen a 1 was also detected in octopus (*Octopus vulgaris*) [32].

NEMATODE TROPOMYOSINS

The prevalence of allergic diseases in rural Africa is very low, and it is hypothesized to be associated with parasite infections [59]. Interleukin 10 (IL-10) and transforming growth factor (TGF- β) are produced against parasite infections and may play important roles in down-regulating allergic responses [60,61]. It was also reported that treatment with antihelmintics increases the skin test reactivity in Gabon [62]. However, infection with *Ascaris* was found to be associated with an increased risk of asthma in rural China [63]. The potential role of tropomyosin, which is a common allergen between parasites and environmental allergens, will be briefly discussed in this section.

Allergic reactions to the fish parasite, *Anisakis simplex*, have been reported [65,66]. Workers handling fish and fish flour have sensitized themselves by the inhalation of this parasite [66]. The allergens secreted by these parasites are thought to be important [67,68]. However, cross-reactivity between IgE binding proteins from *Anisakis* and other insects or mites have been reported [69,70]. *Anisakis simplex* tropomyosin was cloned and its recombinant protein was produced [71]. However, its allergenicity is weak [72], and other proteins have been described as stronger allergens [73-77].

Some of soil-transmitted nematodes are ingested with vegetables. If infective-stage eggs are swallowed, larvae may hatch in the intestine and migrate to the lungs through the bloodstream. The roundworm (*Ascaris lumbricoides*) for example, breaks into the alveoli, ascends the bronchial tubes, is swallowed again, and then develops into an adult in the small intestine. Other geohelminths, such as hookworms (*Ancylostoma duodenale* and *Necator americanus*) and *Strongyloides stercoralis*, also use the pulmonary system to become mobile as larvae. During these passages, the worms may come in contact with the host's immune system and sensitize the host. Therefore, it may be reasonable to investigate the correlations between allergens from geohelminths and inhalant allergens from mites and other domestic arthropods. The IgE reactivities of allergenic tropomyosins are worthy of much attention and study. More than 50% of IgE binding frequencies to tropomyosins have been reported from developing countries, such as in African [78] and Brazilian [79] populations, where a high rate of parasite infections have been reported. However, 5-16% of IgE binding frequencies have been observed in developed countries, where low rates of parasite infection is expected, such as in American [80] and European [81] populations.

TROPOMYOSINS AS INHALANT ALLERGENS

Many species of arthropods are known to be the sources of potent allergens that sensitize and elicit IgE-mediated allergic reactions in humans [82]. Tropomyosin is a well-characterized food allergen, but as a respiratory allergen its characteristics have been poorly examined. Several tropomyosins from domestic arthropods have been reported to be allergenic; however, it is uncertain whether their allergenicities are due to cross-reactivity or poly-sensitization. Tropomyosin levels in house dust could directly reflect the rate of arthropod infestation because all invertebrate animals have tropomyosin, and it is not an excreted allergen such as mite

group 1 [83] or group 2 [84], but it should still be contained in dead debris.

MITE TROPOMYOSINS

Tropomyosin derived from house dust mite (*Dermatophagoides farinae*) is named Der f 10, and was described as a major allergen in Japan [85]. Tropomyosin from another house dust mite (*D. pteronyssinus*) was also cloned and designated as Der p 10 [81]. The IgE binding frequency of Der p 10 was 5.6% in Spain, which contrasts with that of Der f 10. A clone coding for tropomyosin from storage mite (*Blomia tropicalis*), which is frequently found in tropical and subtropical regions, was isolated using mouse anti-Der p 10 antibodies [86]. Its sensitization rate was between 20% and 29%, which were measured by a skin prick test and an enzyme-linked immunosorbent assay (ELISA), respectively. Inhibition experiments also showed that Blo t 10 had unique IgE binding epitopes, even though it shared 95% amino acid sequence identity. A tropomyosin clone was isolated by the screening of a phage display dust mite (*Lepidoglyphus destructor*) library with sera from dust mite-sensitized individuals [87]. Its recombinant protein showed 13% (18/136) IgE binding frequency. A cDNA sequence-encoding storage mite (*Tyrophagus putrescentiae*) was also cloned, and its recombinant protein showed 12.5% (5/40) IgE reactivity in sera from storage mite-sensitized patients (manuscript in preparation). In addition, analysis of sheep scab mite (*Psoroptes ovis*) showed that tropomyosin, along with paramyosin and vitellogen/apolipoprotein, were immunodominant allergens of the sheep scab mite to the animals [88].

COCKROACH TROPOMYOSINS

Tropomyosin was cloned from the American cockroach (*Periplaneta americana*) and named Per a 1 (isoform Per a 7.0101) [42]. The purified tropomyosin could recognize 41% (12/29) of IgE-reacting antibodies from the sera tested. A cross-reactive allergen, putative Bla o 7, from the Oriental cockroach (*Blatta orientalis*), was also detected by an inhibition study. An isoform of the American cockroach tropomyosin Per a 7.0102 was cloned and its recombinant protein reacted with 50% of the sera from Brazilian patients allergic to cockroaches on plaque immunoassay [79]. A cDNA sequence encoding for the German cockroach tropomyosin, Bla g 7, was also obtained [89] and its recombinant protein was produced [90]. Its recombinant or native protein showed 16-18% IgE reactivity to the sera tested. Dusky-brown cockroach (*P. fuliginosa*) tropomyosin was also cloned by RT-PCR [91]. Interestingly, its deduced amino acid sequence was identical with the American cockroach tropomyosin Per a 7.0101, although its nucleotide sequence showed 98.5% identity. Among the sera tested, 26-39% had specific IgE for the recombinant Per a 7.0101.

TROPOMYOSINS FROM OTHER INSECTS

It was reported that fruit flies (*Drosophila melanogaster*) could elicit the allergic disease, especially to the scientists working in laboratories [92]. The pioneering study by Leung *et al.* (1996) on cross-reactivity of invertebrate tropomyosins identified IgE-reactive tropomyosin in the fruit fly [11]. The housefly (*Musca domestica*) could also develop these allergic symptoms [93,94]. A study on various domestic insects

has identified a dense IgE binding component of 37 kDa in size by immunoblotting [95]. The presence of a common allergen in extracts from different species indicates the possible cross-reactivity among different insect species. Further investigation using polyclonal antiserum anti-chicken muscle tropomyosin confirmed the involvement of tropomyosin in the allergic sensitization of patients to household arthropods and its pan-allergenic character [12]. However, it was also reported in a case study on housefly allergy that respiratory sensitization to insects may be highly specific and not cross-reactive [96]. A large number of non-biting midges emerge in the vicinity of standing water. Midge antigens derived from the dead debris from these ephemeral flies may accumulate in air, soil, and dust [97]. Hemoglobin of chironomid larvae has been identified as the most important allergen found in midge-asthmatic patients [98]. Recently, analysis of adult midge has identified tropomyosin (Chi k 10) as a major allergen [99,100].

Atopic workers handling locusts such as *Schistocerca gregaria*, *Locusta migratoria*, and *Melanoplus sanguineus* may develop occupational asthma or other allergic disorders [101-103]. The swarms of locusts may cause serious asthma morbidity and mortality in African countries. A study by Leung *et al.* (1996) indicated that tropomyosin was an important grasshopper (*Mecopoda elongata*) allergen [11]. Novel allergens with molecular weights of approximately 70 kDa from the wings of *L. migratoria* were identified in a recent study [104].

Other insects such as silverfish could be another source of indoor allergens [95]. Cross-reactivity was found to exist between house dust mite and silverfish, cockroach, or chironomid by a RAST inhibition study in the Netherlands [105]. High levels of tropomyosin in dust samples were reported from the Netherlands, and silverfish was described as one of the important sources of dust tropomyosin [106]. Recently tropomyosin (Lep s 1) was cloned from silverfish (*Lepisma saccharina*) and its allergenicity was investigated using its recombinant protein [107]. Recombinant Lep s 1 was able to induce up to 54% histamine release from basophils of allergic patients. It also showed significant cross-reactivity with tropomyosins from cockroach (Per a 7), house dust mite (Der p 10), and shrimp (unidentified species). There might be other allergenic components, and they remain to be investigated [108].

CROSS-REACTIVITY AND IGE-BINDING EPITOPES OF INVERTEBRATE TROPOMYOSINS

The exposure and sensitization to a shrimp tropomyosin may lead to sensitization to certain respiratory allergens [9]. The sensitization to mite tropomyosin may also induce a food allergy [52,109]. An investigation of orthodox Jews, who are strictly prohibited from eating shellfish according to the Kosher dietary laws, suggested that sensitization to inhalant allergens such as mite could result in the sensitization to shrimp tropomyosin [44]. Immunotherapy for house dust mite allergy may also lead to sensitization to tropomyosin, and may result in a food allergy [52,110]. This is of particular significance because immunotherapy is the only known curative way to treat allergic disorders [111,112].

Two IgE-binding regions of Pen i 1 were identified by analyzing IgE reactivities to nine tryptic fragments of Pen i 1

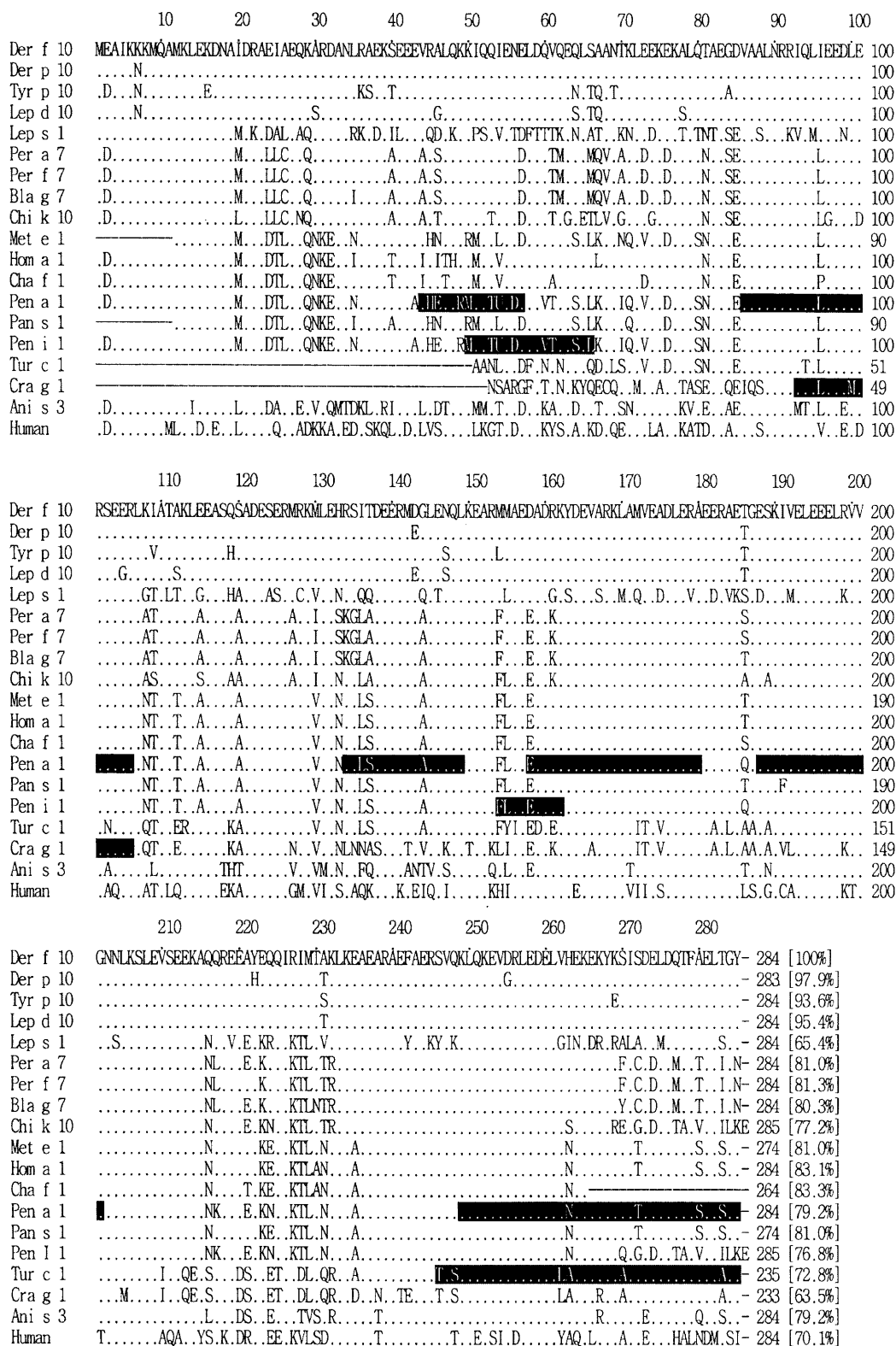


Figure 3. Multiple sequence alignment of allergenic tropomyosins and human tropomyosin: Tyr p 10 (*Tyrophagus putrescentiae*, AY623832), Lep d 10 (*Lepidoglyphus destructor*, AJ250096), Der f 10 (*Dermatophagoides farinae*, D17682), Der p 10 (*Dermatophagoides pteronyssinus*, AF016278), Lep s 1 (*Lepisma saccharina*, AJ309202), Per a 7 (*Periplaneta americana*, Y14864), Per f 7 (*Periplaneta fuliginosa*, AF454866), Bla g 7 (*Blattella germanica*, AF260897), Chi k 10 (*Chironomus kiiensis*, AJ012184), Met e 1 (*Metapenaeus ensis*, U08008), Hom a 1 (*Homarus americanus*, AF034953), Cha f 1 (*Charybdis feriatus*, AF061783), Pen a 1 (*Penaeus aztecus*, DQ151457), Pan s 1 (*Panulirus stimpsoni*, AF030063), Pen i 1 (*Penaeus indicus*, 20), Tur c 1 (*Turbo cornutus*, 5), Cra g 1 (*Crassostrea gigas*, AF239173), Ani s 3 (*Anisakis simplex*, Y19221), and human (*Homo sapiens*, NM001018005). The amino acid sequences of Pen i 1 and Tur c 1 were obtained directly from the articles. The percentage of each sequence identity is given in the parentheses. The shaded areas indicate IgE-binding regions.

[5]. All IgE binding regions identified to date are indicated in Fig. 3. Peptides 6 and 9 corresponding to amino acid residues 153-161 and 50-66, respectively, were able to inhibit 50% of the Pen i 1 specific IgE binding. Four IgE-reactive peptides (E2, 167-179; E3, 136-148; E4, 262-282; E6, 157-169) were identified using a peptide library derived from a *Penaeus aztecus* tail muscle cDNA library. The systematic analysis of IgE binding epitopes of Pen a 1 using 46 synthetic overlapping peptides, which were 15 amino acids long, have identified five major allergenic regions [113]. Each region (region 1, 43-57; region 2, 85-105; region 3, 133-148; region 4, 187-202; region 5, 247-284) was positioned at regular intervals of approximately 42 amino acids (seven heptads), suggesting a relationship with the coiled-coil structure of tropomyosin. Similar regions were recognized as IgE-binding epitopes from *Turbo cornutus* [55] and *Crassostrea gigas* [114]. The C-terminal region (245-284) was noticed as IgE binding epitopes from 18 tryptic digests of Tuc c 1. Cra g 1 was digested with lysylendopeptidase and the IgE reactivity was assessed by ELISA inhibition. Among the 21 peptide fragments, K21 (92-105) showed the strongest inhibition. In addition, the IgE recognition by shrimp-allergic individuals of identical or similar amino acid sequences homologous to Pen a 1 epitopes in lobster, mite, and cockroach are suggested as the basis of cross-reactivity among invertebrate species [115].

Comparisons of IgE epitope regions among mollusk tropomyosins showed the presence of polymorphic sites, indicating that the oyster epitope is species-specific [58]. The presence of Blo t 10-specific epitope was also described [86]. Analysis of IgE binding reactivity to the recombinant fragments of Bla g 7 revealed heterogeneous responses [116]. These observations suggest that genetic and other environmental factors are also important for the development of allergic diseases even for the same allergen [117,118].

Investigation has also indicated that the injection of house dust mite extract for immunotherapy does not seem to induce de novo tropomyosin sensitization [119]. However, diagnostic tests using tropomyosin may be used to select patients with a broad cross-reactivity who may be less suitable for immunotherapy and also to detect novel IgE sensitization possibly induced in subjects undergoing immunotherapy [120].

Based on the accumulated information on IgE binding epitopes, a mutant allergen (hypo-allergen) could be produced and applied as an allergen-specific immunotherapy [121]. The possibility of allergen-specific immune response modulation by targeting tropomyosin to macrophages via a scavenger receptor was explored by maleylation for the treatment of allergy induced by tropomyosin [122].

CONCLUDING REMARKS

Tropomyosin derived from invertebrates has been recognized as a highly cross-reactive allergen. Moreover, tropomyosin could elicit allergic responses by the sensitization of three different routes, such as ingestion, inhalation, and parenteral administration.

Tropomyosin in almost all invertebrate animals appears to be allergenic. A large amount of evidence has been accumulated on their cross-reactivities. It is highly conserved and

a slight structural difference could have an influence on allergenicity. Investigations on allergenic tropomyosins will continue to provide insight on the relationship between structure and allergenicity.

Invertebrate tropomyosin could provide a molecular model to investigate the genetic and environmental factors affecting sensitization and the onset of allergic diseases.

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