

Tropomyosin: An Invertebrate Pan-Allergen

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Key Words

Invertebrate allergens · Crustacea · Cockroach ·
House dust mites · Tropomyosin · Food allergy

Abstract

Among food allergens, crustaceans, such as shrimp, crab, crawfish and lobster, are a frequent cause of adverse food reactions in allergic individuals. The major allergen has been identified as the muscle protein tropomyosin. This molecule belongs to a family of highly conserved proteins with multiple isoforms found in both muscle and nonmuscle cells of all species of vertebrates and invertebrates. Its native structure consists of two parallel alpha-helical tropomyosin molecules that are wound around each other forming a coiled-coil dimer. Allergenic tropomyosins are found in invertebrates such as crustaceans (shrimp, lobster, crab, crawfish), arachnids (house dust mites), insects (cockroaches), and mollusks (e.g. squid), whereas vertebrate tropomyosins are nonallergenic. Studies of cross-reactivities among crustaceans and the high degree of sequence identity among them suggest that tropomyosin is probably the common major allergen in crustaceans. Furthermore, immunological relationships between crustaceans, cockroaches and housedust mites have been established and may suggest tropomyosin as an important cross-sensitizing pan allergen.

Introduction

In recent years, the application of molecular biology techniques to the study of allergens has allowed the characterization of the primary structure of many allergens. Some of these allergens, initially identified in a particular species, appear to be present with certain variations in seemingly unrelated animal or plant species. Cross-reactivity between different allergens, occurs because of shared similar or identical IgE-binding epitopes; thus, these shared epitopes may be the origin of the sensitization and development of allergic symptoms to allergens found in closely or distantly related species. Although most cross-reacting allergens are similar in structure, some differ substantially in molecular configuration. In the last few years, some cross-reacting allergens of foods and inhalants, specially of plant origin, have been investigated. Proteins such as profilins, first identified as an allergen in birch pollen (Bet v 2), are also present in other plant and fruit species and have been implicated as the cause of multiple sensitization to vegetable foods in some patients [1]. Additionally, hevein (Hev b 6.02) from *Hevea brasiliensis* latex has been identified as a major cross-reacting allergen with fruits such as avocado in subjects with latex allergy [2]. Plants may also contain other common proteins such as pathogenesis-related proteins, that are induced by stress conditions [3], and theoretically may be responsible for the cross-reactivity detected among vegetables. However, the cross-reactivity between food and inhalant allergens of animal origin has been far less studied. One example, alpha-livetin, identified as the chicken serum albumin, is the cross-reacting protein responsible for the

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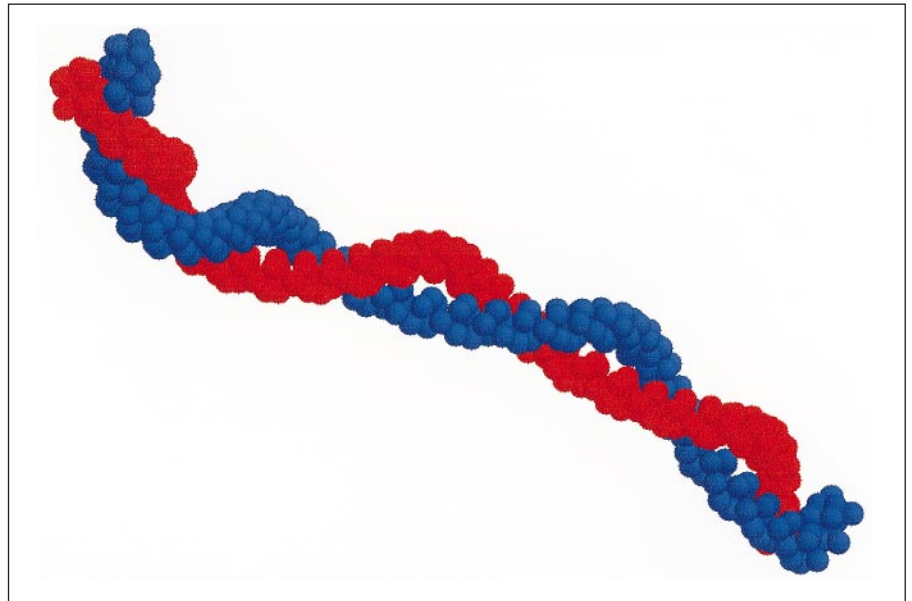


Fig. 1. Three-dimensional, coiled-coil structure of rabbit tropomyosin at 15Å level, rendered as alpha carbon trace.

bird-egg syndrome. In this syndrome, egg allergy is associated with hypersensitivity to feathers and bird excrements in adults [4].

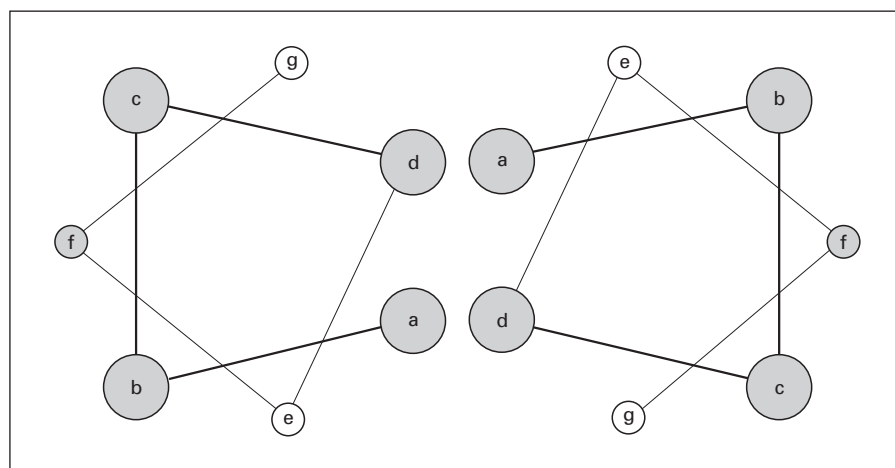
Among food allergens of animal origin, shellfish are a frequent cause of adverse food reactions in hypersensitive individuals [5, 6]. Most shellfish species that are known to elicit allergic food reactions belong to the class crustacea and include shrimp, crab, crawfish and lobster, with the genus *Penaeus* being one of the most frequently reported causes of allergic reactions. For this reason, shrimp has been thoroughly studied; its only major heat-stable allergen has been identified as the shrimp muscle protein tropomyosin [7]. Tropomyosin belongs to a family of highly conserved proteins with multiple isoforms found in both muscle and non muscle cells of all species of vertebrates and invertebrates. Tropomyosin has been suggested as a cross-reacting allergen. It is established that patients allergic to shrimp may also be allergic to other crustaceans and mollusks [8] and some evidence suggests that shrimp may cross-react with non edible arthropoda such as house dust mites [10] (Arachnida) and insects including chironomids [9], cockroaches [10–12], and grasshopper and fruit fly [13]. Tropomyosin may therefore be the basis for cross-reactivity between foods and aeroallergens of animal origin. A comprehensive review of the tropomyosin as a food and inhalant allergen will be presented, as well as a discussion of the cross-reactivities reported in distant species that make tropomyosin a potentially important invertebrate pan-allergen.

Structure of the Tropomyosin Molecule

Tropomyosin belongs to a family of proteins associated with the thin filament in muscle, and microfilaments in many nonmuscle cells. Together with actin and myosin, tropomyosin plays a functional role in the contractile activities of these cells. The function of tropomyosin in nonmuscle cells is not well understood, but it is generally believed to participate in the regulation of cell morphology and motility.

In muscle, two parallel alpha-helical tropomyosin molecules are wound around each other forming a coiled-coil structure [14] (fig. 1). It associates with one complex of troponin and with actin in the thin myofilaments of striated, and cardiac muscle regulating the calcium-sensitive interaction of actin and myosin. The tropomyosin filaments, composed of head-to-tail aggregates, span a length of seven actin monomers in striated muscle while in nonmuscle cells the length is shorter spanning 6 monomers [14]. Traditionally, coiled coils have been identified by the occurrence of a heptad repeat (abcdefg)_n in which generally hydrophobic nonpolar residues occur at positions a and d, while positions b, c, e, f and g are usually occupied by polar or ionic amino acids [14]. The interaction between two alpha-helices in a coiled coil involves these hydrophobic residues in positions a and d [15]. Also charge-charge interactions between acidic residues found in position e and basic residues in position g help also stabilize the coiled-coil. Outer positions b, c, f, must be free to interact with proteins such as actin and troponin (fig. 2).

Fig. 2. Schematic end-on view of coiled coil showing position of residues in the heptad repeat.



Tropomyosins are a group of proteins present in all eukaryotic cells, which contain different isoforms in muscle (skeletal, cardiac and smooth), brain, fibroblasts, platelets and many other non muscle cells. Although the tropomyosins from different tissues are highly homologous, structural differences do exist among these isoforms. For example, at least 12 isoforms have been identified in the rat on the basis of amino acid sequence differences [16]. Some divergent regions among isoforms in smooth and striated muscle seem to correspond to troponin- and actin binding regions or sequences involved in the head-to-tail polymerization [16]. The difference in amino acid sequences in smooth and striated muscle tropomyosins may be related to the mechanism of contraction in the different cell types. In fact, smooth muscle and nonmuscle cell tropomyosins are not regulated by the interaction of tropomyosin with the troponin complex and in them, the interaction of actin and myosin is mainly regulated by the phosphorylation of myosin [16, 17]. Therefore, structurally and functionally different isoforms of tropomyosin are required for the regulation of contractility in the different cell types. A computer search of the gene protein bank yielded the sequences of over 86 types of tropomyosin from different species and organs. Even though they are present in phylogenetically very distantly related species, the degree of homology and functional similarity is in general very high among them. A selection of some vertebrate and invertebrate tropomyosins is represented in table 1 and figure 3, showing amino acid sequence identities and similarities among them.

Allergenic Tropomyosins

Tropomyosin was demonstrated as a major allergen in shrimp by several laboratories [7, 18, 19]. More recently, studies have suggested that tropomyosin is a major allergen in dust mite [20], cockroach [21, 22], lobster [23], squid [24] and other mollusks [13]. Indeed, cross-reactivity between cockroach and mites, nematodes and crustacea has been attributed to the tropomyosin molecule [25].

Shrimp Tropomyosin (Pen i 1, Pen a 1, Met e 1)

Among the food allergens of animal origin, shellfish are a frequent cause of food allergy; allergic individuals can develop urticaria, angioedema, gastrointestinal symptoms, asthma and, in the most severe cases, life-threatening anaphylaxis. Shellfish species which elicit allergic food reactions belong to two phyla: Arthropoda and Mollusca. Edible, allergenic arthropods belong to the class crustacea and include shrimp, crab, crawfish and lobster; species of the shrimp genera *Penaeus* and *Metapenaeus* are the most thoroughly studied crustacea allergens. Hoffman et al. [26] first isolated and partially characterized a heat-stable allergen in shrimp. Termed shrimp antigen II, this glycoprotein had a molecular weight of 38 kD and an isoelectric point of 4.5. Antigen II was shown to be a major shrimp allergen since sera from all 11 shrimp-allergic subjects had a positive RAST for this allergen. Nagpal et al. [27] isolated two heat-stable shrimp allergens from *Penaeus indicus*, called Sa-I and Sa-II. Sa-II had a molecular weight of 34 kD similar to that of shrimp antigen II. The number of amino acid residues of SA-II was estimated at 301 amino acid residues, based on the molecular weight of 34 kD and the amino acid composition. Analysis of allergens of *Penaeus aztecus* (brown shrimp) meat or cooking water by SDS-PAGE and

	Pen a l		Met e l		Hom a l		HomaTMs		Pan s l		Per a 7		Der f 7		Der p 10		DromTM		CravTM		ChlnTM		MyteTM		MytgTM		PatyTM		HalrTM		HelaTM		OncvTM		TricTM		SchmTM		ClosTM		GalgeTM		GalgbTM		SaltTM		OryccTM	
	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si	Id	Si				
Met e l	99	99																																														
Hom a l	98	98	98	98																																												
HomaTMs	93	96	93	96	94	97																																										
Pan s l	98	98	98	98	98	98	94	96																																								
Per a 7	82	90	82	90	81	90	82	90	82	90																																						
Der f 10	81	89	81	90	82	90	83	91	81	90	80	89																																				
Der p 10	81	89	81	90	81	90	82	91	81	90	80	89	98	99																																		
DromTM	77	87	77	87	77	87	79	87	77	87	83	93	75	87	75	87																																
CravTM	67	83	67	83	66	83	66	83	67	83	66	82	66	81	65	81	63	82																														
ChlnTM	37	76	37	76	38	76	38	76	38	76	36	76	39	77	39	77	34	75	78	86																												
MyteTM	37	75	37	75	37	75	37	75	37	75	37	74	36	73	36	73	33	73	83	91	69	83																										
MytgTM	37	76	37	75	37	75	37	75	37	75	37	76	37	75	37	73	36	73	33	74	84	91	69	83	99	99																						
PatyTM	37	76	37	76	37	76	38	76	37	76	36	76	60	77	39	76	34	75	79	86	95	98	70	83	71	83																						
HalrTM	61	79	61	79	61	79	60	79	61	79	60	80	62	78	62	78	55	80	86	93	68	83	71	86	72	87	69	82																				
HelaTM	63	80	63	81	64	80	63	79	64	81</																																						

immunoblotting, identified allergens with molecular weights ranging from 16 to 166 kD [7]. One protein of 36 kD that was recognized by 82% of the patients was designated as the major shrimp allergen Pen a 1 [7]. In this study, no other allergens were detected by more the 25% of the shrimp-allergic population tested making Pen a 1 the only major allergen. In addition, Pen a 1 inhibited 85% of patients' IgE RAST reactivity to whole body shrimp extract indicating that Pen a 1 is responsible for most of the allergenic activity of shrimp [28]. The amino acid compositions and molecular weights of Pen a 1, shrimp antigen II, and Sa-II were remarkably similar, suggesting identical or similar allergens

[7, 29]. Daul et al. [29] first identified Pen a 1 as the shrimp muscle protein tropomyosin based on the amino acid sequence homology of a HPLC-purified 21-residue peptide obtained by endopeptidase Lys-C digestion of Pen a 1; significant homology (60–87%) with tropomyosins from various species was observed. The greatest homology (87%) was with tropomyosin from the fruit fly (*Drosophila melanogaster*), reflecting the phylogenetic relationship between these two arthropods. At the same time, Shanti et al. [18] isolated the major heat-stable allergen from Indian shrimp *Penaeus indicus* (later named Pen i 1), and identified it as tropomyosin by sequencing purified peptides of various

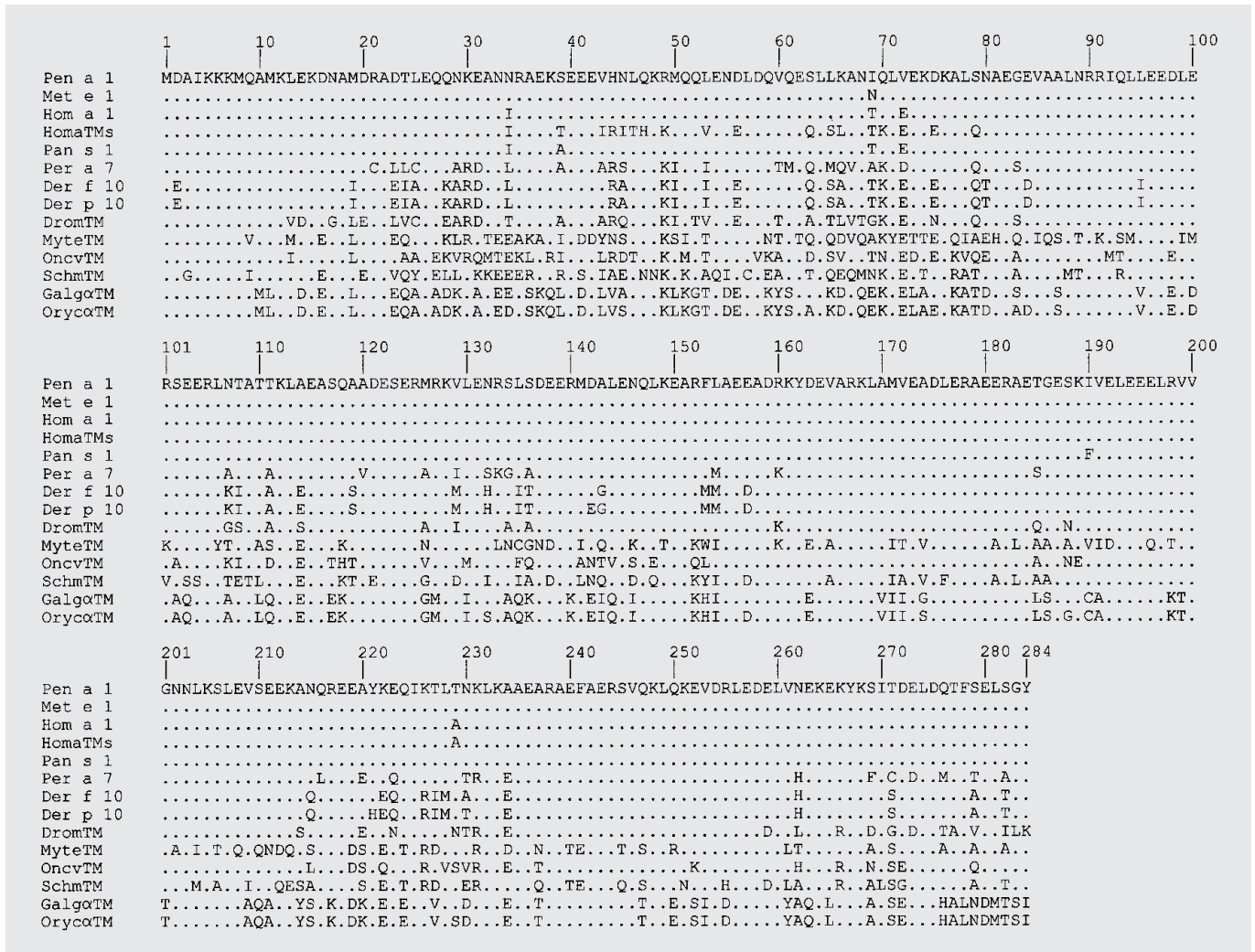


Fig. 3. Amino acid sequence alignments of allergenic and non allergenic tropomyosins. Crustacea: *Penaeus aztecus* (brown shrimp, Pen a 1), *Metapenaeus ensis* (greasy-back shrimp, Met e 1), *Homarus americanus* (Atlantic lobster, Hom a 1), *H. americanus* slow muscle tropomyosin (HomaTMs), *Panulirus stimpsoni* (spiny lobster, Pan s 1). Insecta: *Periplaneta americana* (American cockroach, Per a 7), *Drosophila melanogaster* (fruit fly, DromTM). Arachnida: *Dermatophagoides pteronyssinus*, *D. farinae* (house dust mites, Der p 10, Der f 10). Mollusca: *Mytilus edulis* (blue mussel, MyteTM), Nematoda: *Onchocerca volvulus* (OncvTM), Trematoda: *Schistosoma mansoni* (SchmTM). Vertebrata: *Gallus gallus* (chicken, alpha-tropomyosin GalgaTM), *Oryctolagus cuniculus* (rabbit, OrycaTM) (GenBank data).

proteolytic digests of Sa-II [18]. Another allergenic shrimp tropomyosin, Met e 1, from *Metapenaeus ensis* has been cloned and sequenced by Leung et al. [19].

Other Allergenic Crustacean Tropomyosins Cha f 1, Pan s 1, Hom a 1

Previous immunoblot studies performed with monoclonal antibodies raised against Pen a 1 and sera from shrimp-allergic subjects suggested that tropomyosin is an allergen shared with lobster, crab and crawfish [29]. Indeed, recent-

ly, tropomyosin has been identified as the major allergen in crab and lobster. The screening of a cDNA library constructed from the common crab *Charybdis feriatius* yielded an IgE-reactive clone, designated Cha f 1 [30]. This clone expressed a 264-amino-acid polypeptide that showed significant homology (>90%) with other crustaceans tropomyosins. The 34-kD recombinant protein reacted with IgE of most crab-allergic individuals and absorption of allergic sera with Cha f 1 removes IgE reactivity to crab extract. Also, total inhibition of the IgE reactivity to Cha f 1 was ob-

tained when the sera were preincubated with recombinant Met e 1 illustrating that crab and shrimp tropomyosin are highly cross-reactive and have virtually identical allergenic potential. These findings support previous results which also showed that most of the shrimp specific IgE is directed toward tropomyosin [28, 29]

The tropomyosins of spiny lobster *Panulirus stimpsoni* and American lobster *Homarus americanus* were characterized recently. A cDNA library from spiny lobster was constructed and screened for IgE-reactive clones with sera of crustacean allergic subjects [23]. The expressed 60-kD fusion protein was recognized by serum IgE of allergic subjects. The homology in the sequence of amino acids of Pan s 1 with Met e 1 was higher than 94%. In crustaceans, two types of tropomyosin, fast and slow, have been identified based on the speed at which the muscles from which they originate contract. Mykles et al. [31] cloned and expressed recombinant fast muscle tropomyosin from American lobster *H. americanus*. Immunoblot analysis testing for IgE antibody reactivity binding from crustacean allergic sera identified this protein designated as Hom a 1, a major lobster allergen [23]. Pan s 1 and Hom a 1 are almost identical; they have 97.5% homology. In inhibition experiments the IgE antibody reactivity to Hom a 1 was almost completely inhibited by recombinant Hom a 1, Pan s 1, and Met e 1 [19, 23, 30]. These data taken together show that shellfish-allergic subjects recognize tropomyosin as the major allergen in different crustacean species; this may be the basis for clinical cross-reactivity to different crustacea species.

Allergenic Mollusk Tropomyosins Cra g 1, Tur c 1, Tod p 1

Cra g 1 and Tur c 1 are the major allergens in the oyster *Crassostrea gigas* [32] and in the gastropod *Turbo cornutus* [33], respectively. They were recognized by serum IgE of 3/3 mollusk- and crustacean-allergic subjects. Both allergens have been isolated by a combination of gel filtration, anion-exchange fast protein liquid chromatography (FPLC) and reverse-phase high-performance liquid chromatography. Eight peptides obtained by digestion of Tur c 1 with lysylendopeptidase and trypsin were sequenced, and their partial amino acid sequence was determined. Similarly, sequence information was obtained from ten peptides generated by lysylendopeptidase digestion of the oyster allergen Cra g 1. The homology of Cra g 1 with tropomyosins of the mussel, *Mytilus edulis* (76%), and the abalone, *Haliotis rufescens* (74%), and also the high homology of Tur c 1 with *H. rufescens* (95%), identified both allergens as the muscle protein tropomyosin.

A 38 kD IgE-binding protein designated as Tod p 1 was purified by column chromatography from Pacific squid, *To-*

darodes pacificus. IgE antibody binding to the isolated squid protein was demonstrated by immunoblot with a pool of sera from four squid-allergic subjects. Five peptides obtained by enzymatic digestion of Tod p 1 were sequenced; the greatest homologies of these peptides occurred with tropomyosin from the snail, *Biomphalaria glabrata* (42–100%) as well as with Met e 1 (40–85%), clearly identifying Tod p 1 as another allergenic tropomyosin [24].

Arachnid Tropomyosins (Der p 10, Der f 10)

The major allergens of from house dust mites (*Dermatophgoides pteronyssinus*, *Dermatophagoides farinae*) such as Der p 1 and Der p 2 are well known and have been subject to detailed analysis. More recently, mite tropomyosins, identified as mite allergens (Der f 10 and Der p 10), were cloned and sequenced. Aki et al. [20] cloned and sequenced tropomyosin from *D. farinae*; the deduced amino acid sequence showed high homology with tropomyosins of different animals such as from *D. melanogaster* (76%) or rabbit (58%). The tropomyosin of the closely related mite *D. pteronyssinus*, has been cloned and sequenced by Asturias et al. [22]. The amino acid sequence in this tropomyosin has 98% homology with that of *D. farinae* tropomyosin, and 75–80% with tropomyosins from shrimp and fruit fly. Significant, yet decreased homology was found with mollusks (65%) or vertebrate tropomyosins such as those from man, chicken or mouse (less than 56%). Twenty-five out of thirty-one (81%) mite-sensitive asthmatic subjects demonstrated IgE reactivity to purified Der f 10 [20]. This frequency is comparable with that to other major mite allergens Der f 1 (90%) and Der f 2 (74%), and suggests that Der f 10 is a major mite allergen. Asturias et al. [22] found by western blot analysis that only 4 of 71 (5.6%) mite-monosensitized subjects reacted to recombinant Der p 10. This low incidence of IgE reactivity to Der p 10 in subjects monosensitized to mites does not exclude tropomyosin as a major mite allergen in the general mite allergic population since polysensitized subjects were not included in this second study.

Allergenic Insect Tropomyosin

Another group of arthropods with allergenic species is the class Insecta. Allergens have been described in various species, particularly in German (*Blattella germanica*), American (*Periplaneta americana*) and Oriental (*Blatta orientalis*) cockroaches [34]. Similar to house dust mite tropomyosin, cockroach tropomyosin has only recently been recognized as an allergen. Tropomyosin from the American cockroach *P. americana* (Per a 7) was cloned and sequenced by Arruda et al. [21] by screening a cDNA library from *P. americana* with IgE antibodies from a serum

pool of 14 cockroach allergic asthmatic children. The amino acid sequence deduced from the cDNA was 284 residues long and showed 80, 81 and 82% identity to tropomyosin from the dust mites, *D. pteronyssinus* and *D. farinae*, and from greasyback shrimp, *M. ensis*, respectively. Using individual sera from cockroach-allergic children, 27 out of 53 (51.1%) patients showed IgE binding to cockroach tropomyosin, demonstrating that tropomyosin is a major cockroach allergen.

Concurrently, Asturias et al. [22] have made similar findings; they have cloned and expressed Per a 7. As expected, this protein demonstrated substantial homology with other arthropod tropomyosins from shrimp and mite (80% identity), but much less homology (50% identity) with vertebrate tropomyosin. ELISA and western blot inhibition experiments using natural and recombinant purified tropomyosins from shrimp, mite and cockroach demonstrated IgE antibody cross-reactivity among these allergenic tropomyosins.

Cross-Reactivity among Tropomyosins in Different Species

Cross-Reactivities among Crustaceans

The crustaceans are composed of many edible sea creatures most notably shrimp, crab, lobster, and crawfish; these crustaceans are of particular interest since a number of studies have demonstrated they are major food allergens [35]. Individuals with shrimp hypersensitivity often complain of adverse reactions following ingestion of other shellfish [36]. Since these subjects have positive skin and RAST reactions to crab, crawfish and lobster in addition to shrimp even though they report no exposure to nonshrimp species [36, 37], cross-sensitivity among crustaceans may exist. RAST inhibition and cross-immunoelectrophoresis studies [38–40] were first used to demonstrate cross-reactive allergens among crustacean species that was not due to multiple sensitization. In RAST inhibition studies, shrimp was the most potent inhibitor of the IgE directed to shrimp, followed in potency by crawfish, lobster and crab [39]. More recently, immunoblot studies with sera from shrimp-allergic subjects or with shrimp-Pen a 1-specific monoclonal antibodies demonstrated that Pen a 1 was similar to 36- or 38-kD protein bands in other crustacean species, including blue crab, crawfish, and lobster [13, 29]. Furthermore, sera pre-absorbed with recombinant shrimp tropomyosin lost all of their IgE antibody binding activity to the 38-kD protein in shrimp, crab and lobster [13]. These data taken together clearly demonstrate the presence of cross-reacting, IgE binding epitopes and also provide a rationale for the clinical

hypersensitivity of shrimp-allergic subjects to other crustaceans, even in the absence of prior exposure.

Cross-Reactivities among Crustaceans, Insects and Arachnids

Apart from *Crustaceans*, the arthropods that have been most frequently investigated for cross-reactivity among invertebrates are members of classes *Insecta* and *Arachnida* such as caddis fly, may fly, moth and butterflies, cockroach, non-biting midges (*Chironomidae*) and the reduviid *Triatoma infestans* and *Arachnida* such as house dust and storage mites. Attempts to define allergenic relationships between arthropod allergens, cockroaches in particular and storage or house dust mites (*D. pteronyssinus*, *D. farinae*) have yielded contradictory results [41]. The problem with many of these studies is that cross-reactivity was neither directly determined nor cross-reacting allergens identified. Witteman et al. [10] demonstrated that IgE antibodies of mite-allergic subjects react with silverfish, cockroach and/or chironomid extracts. In fact, 30% of subjects allergic to house dust mite in Netherlands had IgE to these insects. RAST inhibition studies demonstrated cross-reactivity between *D. pteronyssinus* and silverfish, cockroach and chironomid extracts. Alonso et al. [42] demonstrated cross-reactivity between two very common insects in Argentina that cause allergy in indoor and outdoor environments, the cockroach *Periplaneta americana* and the reduviid, *Triatoma infestans*, by RAST and RAST inhibition. Additional studies by Pascual et al. [43] using CAP inhibition and immunoblot inhibition analysis suggested several common IgE-binding components between German cockroach and chironomids.

The cross-reactivity between crustaceans and insects or arachnida has also been widely studied. IgE binding components present in boiled Atlantic shrimp (species not stated) and German cockroach *B. germanica* were shown to cross-react in immunoblotting and CAP inhibition studies done by Crespo et al. [12]; similar studies by O'Neil et al. [11] had previously demonstrated that cockroach antigens cross-react with several species of crustacea. A related study [9] reported that cross-reactivity exists between the larvae of chironomids (nonbiting midges) and shrimp. Also a monoclonal antibody to *D. pteronyssinus* has been described that cross-reacts with an IgE-binding protein (presumably tropomyosin) in crustacea, chironomids and cockroach [10]. Therefore, substantial data confirm the cross-reactivity among different arthropods, in which tropomyosin would play a very important role as a cross-sensitizing allergen. The implications of this cross-reactivity will be discussed below.

Cross-Reactivities with Mollusks

Clinical histories of immediate hypersensitivity to multiple types of crustaceans (decapods such as shrimp, crab and lobster) and mollusks (pelecypodes such as oyster, mussels, scallops and clams) are observed in some shrimp-sensitive subjects [8]. This has prompted several studies in the last years attempting to identify the nature of the cross-reacting allergens [8, 13, 44–46]. Significant RAST reactivity and positive skin prick test to oyster extract has been found in 50% (6/12) crustacean-sensitive or crustacean and (3/7) oyster-allergic subjects [8]. Moreover, RAST inhibition studies showed significant inhibition of the IgE antibody reactivity to oyster with cooked and uncooked shrimp, lobster, crab or crayfish. For unknown reasons, however, the observed cross-reactivity of crustaceans and oyster does not always correlate with clinical sensitivity, suggesting that specific IgE reactivity to mollusk may not always be sufficient for a diagnosis of mollusk allergy.

Leung et al. [13] reported IgE reactivity by immunoblotting to a 38-kD protein present in different mollusks such as Gastropoda (abalone, whelk), Bivalvia (mussel, pen shell, scallop, oyster and clam) and Cephalopoda (cuttlefish, squid, octopus) in 100% (9/9) sera from shrimp-allergic subjects tested. The same sera preabsorbed with recombinant shrimp tropomyosin lost all IgE binding to the 38-kD protein in mollusks. This finding suggests that the 38-kD muscle protein (tropomyosin) in mollusks (squid, oyster and snail) is the major cross-reactive allergen in crustaceans and mollusks.

Cross-Reactivities with Helminths (Nematodes and Trematodes)

Several studies, cited previously have also analyzed the cross-reactivity of arthropods and nematodes. Pascual et al. [43] studied 60 pediatric patients with specific IgE to *Anisakis simplex*. Twenty one sera with specific IgE to both *A. simplex* and German cockroach *B. germanica* were tested for IgE reactivity by immunoblot, immunoblot inhibition, CAP and CAP inhibition to determine whether the association of sensitization to nematodes and arthropods was due to immunological cross-reactivity or multiple sensitization. Immunoblot of *Anisakis* was partially inhibited (mainly reactivity to proteins with molecular weights less than 41 kD) with the red mosquito larvae *Chironomus* spp. and German cockroach extracts. Maximal inhibition of the reactivity to German cockroach was obtained with *A. simplex* while cockroach inhibited only partially the reactivity to *A. simplex*. Similar results were obtained by dose-dependent CAP-inhibition (up to 47%) of the IgE reactivity to cockroach with *A. simplex* antigens. In a related study, Martinez

et al. [25], studied IgE antibody reactivity to a variety of arthropod insect inhalant allergens and the nematodes *A. simplex* and *Ascaris suis*. A polyclonal rabbit anti-chicken tropomyosin demonstrated that the component to which the IgE antibodies reacted was tropomyosin suggesting the presence of allergenic cross-reacting tropomyosins in a variety of insects, mites, crustaceans, mollusks and parasites. However, the observations require further study since allergenic cross-reactivity was only indirectly suggested and the only way to definitely demonstrate cross-reacting IgE antibodies is by inhibition studies.

Cross-Reactivities with Non-Allergenic Vertebrate Tropomyosins (Mammals and Aves)

Tropomyosin is present in muscle and non muscle cells of all vertebrate and invertebrate species; the sequence identities among these tropomyosins range from 50 to 60%. However, in contrast to invertebrate tropomyosins, vertebrate tropomyosins are not allergenic. The possible role of tropomyosin as a meat allergen has been systematically analyzed by dot blot and immunoblot analysis [46, 47]. Subjects with a history of allergy to vertebrate meats did not show any IgE binding to commercial beef, pork, rabbit or chicken tropomyosin, although they reacted to a number of other meat proteins. This indicates that tropomyosin is not an important vertebrate meat allergen.

Since most tropomyosins of different origin are highly conserved, cross-reactivity among them could be possible. However, studies of cross-reactivity among vertebrate and invertebrate tropomyosins have yielded different results. As discussed previously, Martinez et al. [25] reported that polyclonal antibodies to chicken tropomyosin recognized a 35-kD protein (presumably tropomyosin) in insects such as moth and spider, indicating epitopes similar in chicken tropomyosin and the 35-kD protein of moth and spider. In contrast, the analysis of the reactivities of shrimp-allergic subjects' IgE to shrimp and homologous mammalian (porcine, bovine, rabbit, chicken or mouse) tropomyosins and their fragments by dot blot and immunoblot analysis [13, 48] demonstrated that Pen a 1-specific IgE antibodies did not cross-react with any of the mammalian tropomyosins or their fragments. The immunoblot analysis showed that all peptides from Pen a 1 digests bound IgE or monoclonal antibodies reactive to Pen a 1 [48]. The lack of in vitro IgE-reactivity to vertebrate tropomyosins in meat-allergic subjects is in agreement with the low frequency of allergy symptoms to meats found in crustacean-allergic subjects. The apparent contradiction of the results of Martinez et al. [25] and those of Reese et al. [50] and Leung et al. [13] may be explained by the different specificities of the

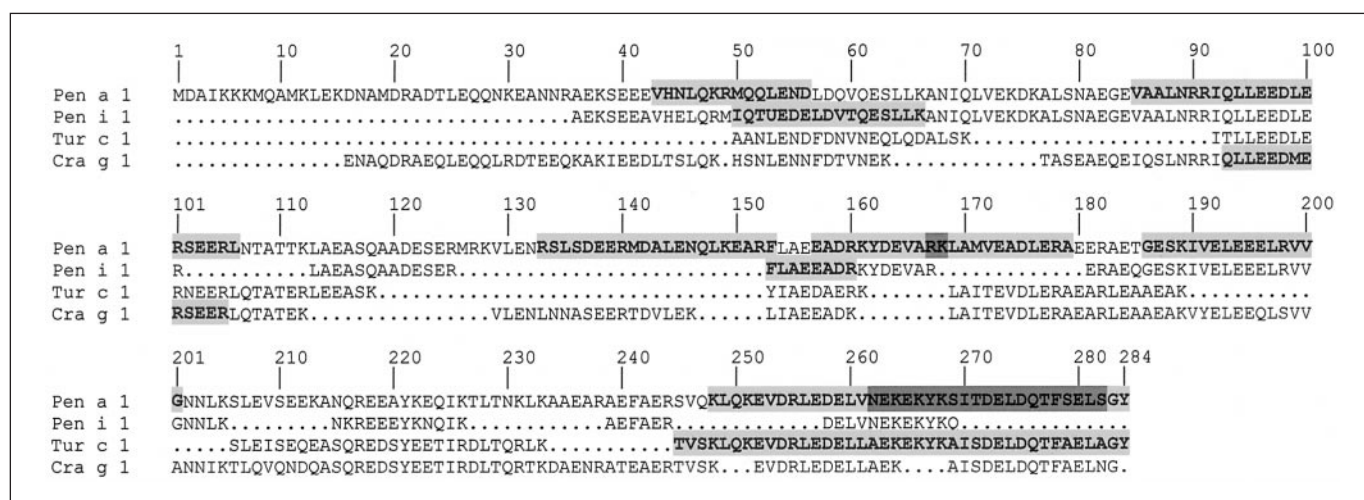


Fig. 4. IgE binding sites on invertebrate tropomyosins Pen a 1 (*Penaeus aztecus*), Pen i 1 (*P. indicus*), Cra g 1 (*Crassostrea gigas*), and Tur c 1 (*Turbo cornutus*). Pen a 1: region 1 (43–57); region 2 (85–105); region 3 (133–153); region 4 (187–201); region 5 (247–284); E2 (167–179); E3 (136–148); E4 (262–282); E6 (157–169). Pen i: 50–66, 153–161. Tur c 1: MT17 (245–284). Cra g 1: K21d (92–105). The IgE-binding regions are highlighted in grey; overlaps between two IgE-binding regions of Pen a 1 are highlighted in dark grey.

polyclonal anti-chicken tropomyosin antiserum and the Pen a 1-reactive monoclonal antibodies.

Cross-Reactivity of Tropomyosin and Bacterial Protein M

An intriguing observation suggests that cross-reactivity between human and bacterial proteins may be responsible for the induction of autoimmune disease. In rheumatic fever, a possible cross-reactivity between streptococcal and human cardiac muscle proteins has been suggested. M proteins are the major virulence determinants of group A streptococci rendering the microorganisms resistant to phagocytosis. Specific antibodies against the streptococcal M protein aid in the opsonization of the bacteria and protect the host against infection. Cross-reactivity between M proteins and the muscle protein tropomyosin has been demonstrated by Fenderson et al. [49] using murine monoclonal antibodies specific for streptococcal M protein. Interestingly, monoclonal antibodies produced to streptococcal membranes cross-reacted with M6, M5 streptococcal proteins and skeletal and cardiac mammalian tropomyosins when tested by immunoblot and ELISA; in most instances, the cross-reactivity also occurred with other coiled-coil proteins such as myosin or actin. Fenderson et al. [49] also reported that the global homology of protein M and mammalian tropomyosin is 18.5%. However, when particular regions are compared, the homology rises to 31%. These cross-reacting epitopes are thought to explain the damage often seen in the heart after acute rheumatic fever in man. It

is not known whether such cross-reactivity between bacterial proteins and invertebrate tropomyosins exists; since a cross-reactivity could be clinically important, studies concerning this cross-reactivity should be performed.

IgE Binding Epitopes of Tropomyosin

Shanti et al. [18] first identified two IgE-binding regions of Pen i 1 by analyzing IgE antibody reactivities of shrimp-allergic patients and control subjects to nine tryptic fragments of Pen i 1. Inhibition of binding of Pen i 1-specific IgE antibodies was maximal (50%) when peptides 6 and 9, consisting of amino acid residues 153–161 and 50–66, respectively, were used as inhibitors. Reese et al. [50] screened a unidirectional expression cDNA library from *P. aztecus* tail muscle with a Pen a 1-specific monoclonal antibody, identifying 4 positive clones. All 4 recombinant proteins were recognized in immunoblot analysis by serum IgE from shrimp-allergic individuals. One of the clones (P7) was purified and used to construct a peptide library (Novatope epitope mapping system). The library was screened with individual serum from shrimp-allergic subjects, identifying four IgE-reactive peptides (E2, E3, E4 and E6) that were between 13 and 21 amino acids long. All 4 peptides were located in the second half of the molecule including the C terminus (fig. 4).

In the first systematic analysis of IgE binding epitopes of Pen a 1, Ayuso et al. [51] synthesized a battery of 46 overlapping peptides, 15 amino acids long, spanning the whole length of the Pen a 1 tropomyosin (284 residues). The peptides were tested for IgE binding with sera of 18 shrimp-allergic subjects. Subjects showed a wide range of individual reactivity patterns; the number of peptides bound by an individual serum ranged from 1 to 15 (mean: 8) peptides. Based on frequency and intensity of IgE binding, 5 major allergenic regions were identified: region 1 (43–57), region 2 (85–105), region 3 (133–153), region 4 (187–201) and region 5 (247–284). The IgE binding sequences occur at regular intervals in the molecule (approximately every 42 amino acids), suggesting some relation with the coiled-coil structure of tropomyosin since major IgE-binding regions are spaced approximately every 6 heptads.

Figure 4 summarizes the IgE-binding regions in different tropomyosin molecules identified thus far. Some of the 5 allergenic regions identified by Ayuso et al. [51] in Pen a 1 have been also recognized as allergenic by other authors in tropomyosins from different origin such as those from the mollusks *Turbo cornutus* [52] and *Crassostrea gigas* [53] or in the shrimp *P. indicus* [18]. Twenty-four peptides (K1–K24) obtained from lysylendopeptidase digestion of Tur c 1 and 18 tryptic digests (MT1 to MT18) of maleyl-Tur c 1 were used to inhibit the binding of IgE antibodies from crustacean- and mollusk-allergic sera to Tur c 1 by ELISA inhibition analysis [52]. No inhibition was obtained with the lysylendopeptidase digests, but substantial inhibition (close to 30%) was noticed with 4 tryptic digests (MT 15, 16, 17 and 18). Sequencing of MT 15 and MT17 identified the C-terminal region as one of the IgE-binding epitopes of Tur c 1. The inhibitory capacity of each one of the 21 peptides (K1–K21) generated by lysylendopeptidase activity on Cra g 1 was also assessed by ELISA inhibition [53]. K21 showed maximal inhibition (34%) of the binding of IgE antibodies from crustacean- and mollusk-allergic subjects to Cra g 1 while the mixture of all 21 peptides inhibited about 70% the binding to Cra g 1. Two tryptic digests of K21, called K21d (spanning amino acid residues 92–105) and K21e retained most of the ELISA inhibitory capacity of K21.

Further analysis of two allergenic regions of Pen a 1, region 3, and 5 by Reese et al. [54] have led to the determination of tropomyosin epitopes (shortest peptide with maximal IgE-binding capacity). In region 3, the size of the individual epitope ranged from 6 to 9 amino acid residues, with the core epitope being Pen a 1 137–141 (DEERM). This sequence was found to be identical to the homologous sequences of American cockroach (*P. americana*) and house

dust mite (*D. pteronyssinus*) allergenic tropomyosins, Per a 7 and Der p 10, respectively. Comparison with vertebrate tropomyosin shows a substitution in position 140 where an arginine (R) is substituted for a lysine (K) residue. In region 5 three epitopes have been identified. All 4 subjects who showed IgE reactivity to region 5 recognized the peptide Pen a 1 (266–273) (KYKSITDE) as the minimal IgE-binding site. This epitope differs from homologous regions of vertebrate and other invertebrate tropomyosins. The second epitope, recognized by 3 out of 4 subjects, is centered around a core, Pen a 1 (251–259). The core is identical for the homologous sequences of Per a 7, Der p 10 and Tur c 1, but differs in three positions from the nonallergenic vertebrate tropomyosins. The third epitope, Pen a 1 (273–281), is only recognized by one subject and does not seem to be an important one. In conclusion, the results demonstrate that individual epitopes have large overlaps and are clustered around a common core sequence. The identity of some of these epitope cores with homologous, allergenic tropomyosins of American cockroach (*P. americana*) and house dust mite (*D. pteronyssinus*) tropomyosin may explain reported cross-reactivities between shrimp and other arthropods. The limited amino acid sequence differences between individual epitopes as well as epitope cores and non-allergenic tropomyosins makes it possible to test the impact of all possible combinations of amino acid substitutions on the IgE binding capacity of the Pen a 1 epitopes.

Clinical Relevance of the Cross-Reactivity

Are the structural and immunochemical similarities of tropomyosins among different species of invertebrates clinically significant? It is particularly intriguing since the type, dose and frequency of exposure (inhalation versus ingestion) of allergic individuals to these different allergens can vary markedly. For example, shrimp sensitivity occurs generally at high dose exposure of allergens under the harsh conditions of the gastrointestinal tract. In contrast, mite and cockroach exposure generally occurs at much lower doses in the more physiologic conditions of the respiratory tract.

Van Ree et al. [55] investigated the sera of 17 patients receiving immunotherapy for house dust mite allergy for IgE antibodies against snail and shrimp, foods that cause allergy in the Netherlands. Although they showed no prior reactivity, 3 of the 17 patients studied developed a positive RAST for shrimp and snail; 2 of the 3 sera contained IgE antibodies against cross-reactive tropomyosin from mite, snail and shrimp. These 2 patients with anti-tropomyosin IgE also had a positive skin prick test for shrimp and demonstrated clini-

cal symptoms (oral allergy syndrome) after eating shrimp. These studies strongly suggest that increased exposure of some patients to mite antigen (through mite immunotherapy) results in sensitization to cross-reacting seafood tropomyosins. The importance of the clinical implications of this cross-reactivity is highlighted by other reports where more than 50% of food allergy to snails [56] and limpet [57] reported in 12 and 6 subjects occurred after a long period of immunotherapy for mites. These studies taken together with those of van Ree et al. [55] raise some concern about the induction of food allergy through cross-reacting allergens in immunotherapy extracts.

In another study, de Blay [1999, unpubl.] reports similar findings through a different approach. He investigated mite and cockroach allergy associated with sensitization to crabs and shrimp. Specific IgE to tropomyosin of mites, cockroach, crab and shrimp were measured. The results indicate that 6 patients became allergic to mite and German cockroach subsequent to their sensitization to crustacea tropomyosin due to unusual ingestion of crabs and shrimp. These studies suggest that exposure and sensitization to a particular food allergen may ultimately lead to sensitization to certain aeroallergens. These results taken together suggest that the in vitro cross-reactivity among tropomyosins in different invertebrate species not only exists, but also has important clinical implications and may result in the induction of sensitization and allergic reactions to both foods and inhalants in the same patient. These studies also help to explain some unusual observations of reactivity in the absence of sensitization such as positive skin tests of shrimp in mite-

or cockroach-allergic orthodox Jews [A. Reshef, G. Sussman, pers. commun.).

There is accumulating evidence that cross-reactivity occurs among members of the class arthropoda, particularly crustaceans, insects and arachnids, and also with other invertebrates such as mollusks and nematodes. The common allergen that accounts for much of this cross-reactivity is the muscle protein tropomyosin. This molecule has a highly conserved structure that is important in muscle contraction. It consists of a coiled-coil structure stabilized by hydrophobic interactions. Because tropomyosin appears to be an allergen in so many invertebrate species, one could speculate that it is a pan-allergen of invertebrates. Molecules with generally minor structural differences yet substantial differences in allergenicity could be very useful for our understanding of the relationship between protein structure and allergenic activity. Since it appears to be such a potent and widespread allergen, identification of structural features that contribute to its allergenicity will help advance our understanding of the relationship of protein structure to allergen function. The understanding of basic mechanisms of allergen function will lead to better methods for the treatment of allergic patients, such as the usage of modified allergens with reduced or abolished IgE-binding capacity.

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