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The Occurrence of Sequences Identical with Epitopes from the Allergen Pen a 1.0102 Among Food and Non-Food Proteins

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The presence of common epitopes among tropomyosins of invertebrates, including arthropods, *e.g.* edible ones, may help to explain the molecular basis of cross-reactivity between allergens. The work presented is the first survey concerning global distribution of epitopes from Pen a 1.0102 in universal proteome. In the group of known tropomyosin epitopes, the fragment with the sequence ESKIVELEEEL was found in the sequence of channel catfish (*Ictalurus punctatus*) tropomyosin. To date, this is the first result suggesting the presence of a complete sequential epitope interacting with IgE in vertebrate tropomyosin. Another fragment with the sequence VAALNRRIQL, a major part of the epitope, was found in 11 fish, 8 amphibians, 3 birds, 19 mammalians and 4 human tropomyosin sequences. Identical epitopes are common in sequences of invertebrate tropomyosins, including food and non-food allergens annotated in the Allergome database. The rare pentapeptide with the DEERM sequence occurs in proteins not sharing homology with tropomyosins. Pathogenic microorganisms are the most abundant category of organisms synthesizing such proteins.

INTRODUCTION

Tropomyosins and their fragments belong to the seafood allergens [Jędrychowski *et al*., 2008; Hajeb & Selamat, 2012; Kumar *et al*., 2012]. Their allergenic properties are usually attributed to invertebrate proteins and peptides derived from them [Kumar *et al*., 2012]. Few years ago vertebrate tropomyosins were regarded as non-allergenic proteins due to their susceptibility to hydrolysis by pepsin (EC 3.4.23.1; ID A01.001 in the MEROPS database of proteolytic enzymes) or absence of epitopes characteristic for invertebrate tropomyosins. On the other hand, the number of protein sequences recorded in databases is growing rapidly and it is possible that the above-mentioned criteria will not be fulfilled by new vertebrate tropomyosin sequences. The first allergenic tropomyosin of vertebrate origin – protein from fish *Oreochromis mossambicus* (Ore m 4.0101, UniProt accession No: K4PEK4, Allergome code 10146) has been recently described [Liu *et al*., 2013] on the basis of experimental results. Some vertebrate tropomyosins (*e.g.* human) are annotated in Allergome database as *in silico* predicted allergens on the basis of their amino acid sequences.

Tropomyosin sequences are highly conserved and some of them were known to contain common sequential epitopes. The presence of a local sequence identity suggests potential cross-reactions between proteins [Kleter & Peijnenburg, 2002; Marti *et al*., 2007; Kanduc, 2008; 2012; Minkiewicz *et al*., 2011]. Examples of invertebrate tropomyosins with common epitopes can be found in the BIOPEP database of allergenic proteins [Dziuba *et al*., 2013]. All tropomyosin entries in this database contain epitopes identical to the protein of *Farfantepenaeus aztecus* (BIOPEP ID 76, code 3929 in Allergome database), which was subjected to extensive epitope mapping [Shanti *et al*., 1993; Ayuso *et al*., 2002; Reese *et al*., 2005]. Identity between epitopes of tropomyosins from various edible invertebrates has also been emphasized by Marti *et al*. [2007], Zheng *et al*. [2011] and Abramovitch *et al*. [2013].

Local sequence identity or similarity may constitute a molecular basis of cross-reaction with the human immune system. Celiac-toxic fragments of wheat gliadins and similar fragments of other proteins may serve as an example of such cross-reactions. Fragments similar to celiac-toxic peptides were found in, among others, sequences of bovine β-casein, maize prolamin, oat and yeast proteins [Darewicz *et al*., 2007]. The presence of the above fragments can be considered as the molecular basis of cross-reactivity between the above-mentioned proteins and wheat gliadins [Cabrera-Chávez & Calderón de la Barca, 2009; Cabrera-Chávez *et al*., 2012; Vojdani & Tarash, 2013] studied *in vitro* using antibodies from celiac patients. In the case of arthropod tropomyosins the consensus sequence constitutes allergenic region [Marti *et al*., 2007]. Some experimental results obtained using tropomyosins suggest that presence of common linear epitopes

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TABLE 1. Bioinformatics tools mentioned in the article.

Name of database or program	Website	Reference
Allergome	http://www.allergome.org/	Mari *et al*. [2009]
BIOPEP	http://www.uwm.edu.pl/biochemia/index.php/pl/biopep	Dziuba et al. [2013]
BLAST	http://www.ebi.ac.uk/Tools/sss/wublast/	Altschul *et al*. [1997]
FAO Fisheries and Agriculture website	http://www.fao.org/fishery/en	
GOR V	http://gor.bb.iastate.edu/	Sen *et al.* [2005]
Human Gut Microbiome Project	http://genome.wustl.edu/genomes/	Hattori & Taylor [2009]
Immune Epitope Database (IEDB)	http://www.immuneepitope.org/	Vita *et al*. [2010]
InterPro	http://www.ebi.ac.uk/interpro/	Hunter *et al*. [2012]
Merops	http://merops.sanger.ac.uk/index.shtml	Rawlings *et al*. [2014]
MisPred	http://mispred.enzim.hu/	Nagy *et al*. [2008]
PATRIC	http://patricbrc.vbi.vt.edu/portal/portal/patric/Home	Snyder *et al*. [2007]
PeptideMass	http://web.expasy.org/peptide_mass/	Wilkins *et al*. [1997]
Protein Data Bank	http://www.wwpdb.org/	Rose *et al.* [2011]; Kinjo *et al.* [2012]; Velankar *et al.* [2012]
ResearchGate	http://www.researchgate.net/.	
Scopus	http://www.scopus.com/home.url	
Tachyon	http://tachyon.bii.a-star.edu.sg/index.action	Tan *et al*. [2012]
UniProt	http://www.expasy.org	The UniProt Consortium [2014]

may lead to cross-reactivity of allergenic proteins. Albrecht *et al.* [2009] have found cross-reactivity between tropomyosin from *Farfantepenaeus aztecus* and mouse tropomyosin with inserted epitopes from the above mentioned allergen. Interactions of hybrid protein with patient's sera were significantly weaker than these of shrimp allergen, but much stronger than these of mouse tropomyosin.

In our previous study [Darewicz *et al*., 2007], fragments with identities exceeding 50% were taken into account. In further studies [Minkiewicz *et al*., 2011; 2012] protein fragments possessing 100% identity with entire epitopes were considered. Pentapeptides are the shortest fragments recognised by the immune system [Kanduc, 2008]. The existence of a common fragment containing at least 6–8 amino acid residues is a recommended bioinformatics criterion defining protein as an allergen cross-reacting with previously-known allergenic proteins [Goodman, 2006]. Protein fragments used usually for epitope mapping have at least 10, and usually 15, amino acid residues. Increasing the length of common fragments suggests increasing the likelihood of cross-reactivity between proteins. Additional restriction is recommended [Dall'Antonia *et al*., 2014]. The secondary structure of peptide should mimic the structure of the corresponding fragment of entire protein.

The aim of this study was to analyse the distribution of peptides considered as experimentally-recognised epitopes of the allergen Pen a 1.0102 in the set of all known protein sequences. The questions to be answered are as follows: Do common fragments occur in invertebrate tropomyosins and in vertebrate tropomyosins? How many invertebrate tropomyosins contain common epitopes forming a possible molecular basis of cross-reactivity? Are epitopic peptides from invertebrate tropomyosins present in non-homologous proteins?

METHODS

Sixty sequences of epitopes from the BIOPEP database [Dziuba *et al*., 2013], attributed to tropomyosin from the shrimp *Farfantepenaeus aztecus* (allergen Pen a 1.0102) were used as query sequences. Most of the epitopes used were also registered in the Immune Epitope Database (IEDB) [Vita *et al*., 2010]. Secondary structure of epitopic peptides was predicted using GOR V program [Sen *et al*., 2005]. The UniProt database [The UniProt Consortium, 2014] was searched using the WU-BLAST program [Altschul *et al*., 1997]. Protein entries in UniProt contain, for example, links to the InterPro domain database [Hunter *et al*., 2012] and to the Allergome database [Mari *et al*., 2009] if they are known allergens. The following parameters were applied: the PAM 10 matrix, expected threshold: 1000, data sorting according to high score [Minkiewicz *et al*., 2012]. The remaining parameters were set at default values. Only the sequences whose identity 100% matched the query sequences or the length of continuous fragment exceeding 8 amino acid residues were taken into account in line with previous recommendations [Goodman, 2006; Minkiewicz *et al*., 2011]. Vertebrate protein sequences annotated as "inferred from homology" or "predicted" were examined using the MisPred program [Nagy *et al*., 2008].

Proteolysis of vertebrate tropomyosins by pepsin and trypsin (EC 3.4.21.4; MEROPS ID S01.151) was simulated using the PeptideMass program [Wilkins *et al*., 1997]. Input data included pepsin specificity at pH=1.3, and up to one missed cleavage was allowed. Proteins containing epitopic peptides were classified according to presence of appropriate domains. Due to progress in integrating other domain databases with InterPro, only this database was used for protein classification. Epitopes located out of domains were also included. Data on particular species was retrieved from the Scopus literature database, Human gut Microbiome Project website [Hattori & Taylor, 2009], PATRIC website [Snyder *et al*., 2007] as well as the FAO Fisheries and Agriculture website.

UniProt database screening was carried out in December 2011. Allergome was accessed in January 2013, GOR V in September 2014 and other bioinformatics tools (Table 1) in February 2014.

RESULTS AND DISCUSSION

Occurrence of common shrimp tropomyosin epitope sequences in vertebrate and invertebrate tropomyosins

A full list of epitope precursors found is available at author's profile at ResearchGate portal or available upon request from the corresponding author.

The epitope with the sequence ESKIVELEEEL (IEDB ID 14182) was found in the sequence of channel catfish (*Ictalurus punctatus*) tropomyosin (UniProt entry name: E3TF21_ICTPU). The location of the above epitope in the protein sequence is presented in Figure 1. Bonds susceptible to pepsin action within the epitope and in the nearest surroundings are also presented in Figure 1. The most likely secondary structure for the above mentioned peptide as well as longer fragments of Pen a 1.0102 allergen and *Ictalurus punctatus* tropomyosin containing its sequence (ESKIVELEEELRV, ESKIVELEEELRVVG, ESKIVELEEELRI and ESKIVELEEELRIVG) is a-helix as judged using GOR V program. The predicted secondary structure of peptides is the same as experimentally found secondary structure of entire tropomyosin chains, presented in the Protein Data Bank.

The second fragment of *Farfantepenenaeus aztecus* tropomyosin in vertebrate proteins is decapeptide VAALNRRIQL, a fragment of the epitope interacting with IgE, possessing sequence VAALNRRIQLLEEDL (IEDB No. 67524). Human and animal tropomyosins containing fragments similar to the epitope (with sequence VAALNRRIQLVEEEL) are summarized in Table 2. Both of these pentadecapeptides possess 13 common amino acid residues, including common N-terminal decapeptide. Changes in the sequence (valine *versus* leucine and glutamic acid *versus* aspartic acid) do not lead to significant changes in physico-chemical properties. The most likely secondary structure predicted for both above pentadecapeptides using GOR V program, is α-helix. The majority of mammal, bird and fish species indicated in Table 2 are sources of food. Only a few proteins were identified at the protein level, but the existence of others has been inferred based on homology with previously-discovered proteins.

It should be taken into account that the application of the strategy described here misses part of allergens as pointed out previously [Minkiewicz *et al*., 2011]. Proteins containing known epitopes are likely to be allergens, but absence of epitopes from a previously known allergenic homolog does not provide evidence that protein is safe. This remark is supported by the fact that the sequence of the allergenic fish tropomyosin Ore m 4.0101 [Liu *et al*., 2013] does not contain epitopes from shrimp tropomyosin. It could not be predicted as an allergen only on the basis of the presence of known epitopes from the set used in this work. On the other hand, there were some premises to take into account possibility of allergenicity of vertebrate tropomyosins before discovery of the Ore m 4.0101.

The presence of experimentally confirmed epitope suggests that channel catfish tropomyosin is potentially allergenic, in line with the previously-proposed decision tree [Minkiewicz *et al*., 2011]. More precisely: this protein may reveal at least interaction with Immunoglobulin E specific for the epitopic peptide ESKIVELEEEL. Sereda *et al*. [2010] have found that there are animal antibodies interacting both with invertebrate and vertebrate tropomyosins *in vitro*. Such property is considered to be one of the three attributes of food allergens [Bannon, 2004]. If protein does not induce allergic sensitiza-

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MDSIKKKMMA  MKLEKENAME  KALNLETQLK  EKANDMDKKE  EEMNEMQTKV   50

KTIQAEVDTV  QESLQEATSK  LEETEKRATN  AEAEVAAMTR  RIRLLEEDLE  100

QSGGRLTDTS  SKLDDASKAA  EESERSRKTL  ETRSISDDER  MAQLEDQVKE  150
                                    ↓           ↓  ↓
AKYIAEDAER  KYDEAARRLA  VTEVDLERAE  SRLETSESKI  VELEEELRIV  200

GNNMKSLEVS  EQESAQREES  YEETIRDLTE  RLKLAEQRAA  EADRQVSKLQ  250

NEVDRLEDEL  LSEKERFRGI  GGELDTTFAE  LTSF
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FIGURE 1. Sequence of channel catfish tropomyosin. The epitope interacting with B cells is underlined. Bonds which are predicted to be susceptible to pepsin hydrolysis and which could induce the release and/or degradation of the epitope are marked with arrows. The bond corresponding to the missed cleavage is indicated in bold.

TABLE 2. Proteins containing a fragment with the sequence VAALNRRIQLVEEEL. Entry names according to UniProt database are given. Asterisks indicate species used as food resources.

Source	Proteins
Human	TPM4_HUMAN; P67936–2 (protein level); B4DTB1_HUMAN; B4DVY2_HUMAN (transcript level);
Mammalian	TPM4_HORSE (*Equus caballus**; protein level); F6ZBW3_HORSE (*Equus caballus**; from homology); TPM4_PIG (*Sus scrofa**; protein level); D0G7F7_PIG (*Sus scrofa**; transcript level); F6WBZ4_ORNAN (*Ornithorhynchus anatinus*; from homology); F7CAE6_CALJA; F7I2I6_CALJA (*Callithrix jacchus*; from homology); G1R039_NOMLE (*Nomascus leucogenys*; from homology); E2R661_CANFA; E2R662_CANFA (*Canis familiaris*; from homology); F7GGJ2_MONDO; F7GGJ7_MONDO (*Monodelphis domestica**; from homology); G1U5H1_RABIT; G1SY36_RABIT (*Oryctolagus cuniculus**; from homology); G1LTE9_AILME; D2GUY3_AILME (*Ailuropoda melanoleuca*; from homology); F7AWG5_MACMU (*Macaca mulatta*; from homology); A6QR15_BOVIN (*Bos taurus**; transcript level); G1PU01_MYOLU (*Myotis lucifugus*; from homology)
Bird's	Q90349_COTCO; Q90348_COTCO (*Coturnix coturnix**; from homology); G1N2D9_MELGA (*Meleagris gallopavo**; from homology)
Amphibian	Q6PF72_XENLA; Q91865_XENLA; Q7SYY4_XENLA; Q91726_XENLA (*Xenopus laevis*; transcript level); Q4F8N9_HYLCH (*Hyla chrysoscelis*; transcript level); Q8QGC3_AMBME (*Ambystoma mexicanum*; transcript level); Q28GF0_XENTR (*Xenopus tropicalis*; transcript level); F6SK00_XENTR (*Xenopus tropicalis*; from homology)
Fish	Q1LXM1_DANRE; Q1LXM2_DANRE; Q5U3J6_DANRE; Q7SXW1_DANRE; Q7T3F0_DANRE (*Danio rerio*; transcript level); F1QKG7_DANRE; F1R412_DANRE (*Danio rerio*; from homology); C1BIJ6_OSMMO (*Osmerus mordax*; transcript level); Q805C4_TAKRU; Q805C5_TAKRU (*Takifugu rubripes**; transcript level); B9EMY1_SALSA (*Salmo salar**; transcript level)

Protein level – protein clearly identified by, for example, sequencing or mass spectrometry. Transcript level – experimental evidence for transcription. From homology – the existence of a protein is likely due to the presence of homologous sequences in closely-related species.

tion or allergic reactions, it is considered to be an incomplete allergen, *i.e.* a non-ellicitor [Bannon, 2004]. On the other hand, it was found that epitopes from shrimp are useful markers in the diagnosis of allergy to tropomyosins [Ayuso *et al*., 2012]. This fact increases the likelihood of channel catfish tropomyosin allergenicity. Assuming that all predicted bonds are hydrolysed, the analysed epitope should be susceptible to pepsin cleavage (Figure 1) and released if at least one bond is resistant to hydrolysis. This finding seems to be the weak point of possible allergenicity prediction because resistance to pepsin hydrolysis is regarded as a criterion of allergenicity [Schnell & Herman, 2009]. On the other hand, bonds that are theoretically predicted to be susceptible to proteolysis could actually be resistant. Examples of decreased protein susceptibility to proteolysis due to interactions with other compounds were presented by Schnell & Herman [2009].

The confirmation of the tested hypothesis would expand the database of vertebrate allergens and the list of potential risk factors for people who are allergic to crustaceans, insects and mites.

The use of epitope sequences as queries in the screening of the UniProt database supported the discovery of the first vertebrate (fish) tropomyosin containing the known epitope interacting with IgE. Although the allergenicity of vertebrate tropomyosins (apart of tropomyosin from *Oreochromis mosambicus*) and the cross-reactivity between vertebrate and invertebrate tropomyosins have not been experimentally confirmed, this possibility cannot be ruled out. The number of identified protein sequences is growing rapidly, therefore it is highly likely that new sequences of vertebrate tropomyosins containing epitopes interacting with IgE or their fragments with more than 8 amino acid residues will be found. Allergenicity predictions may be tested experimentally to verify whether vertebrate tropomyosins and/or their fragments characterised by the highest similarity to tropomyosin epitopes fulfil the following criteria of allergenicity: interaction with immunoglobulin E, induction of allergic sensitization and induction of allergic reactions [Bannon, 2004; Schnell & Herman, 2009]. An additional experiment could be designed to determine whether vertebrate protein fragments which are identical or similar to epitopes are able to survive gastrointestinal digestion.

Occurrence of fragment identical or similar to shrimp epitopes among proteins

The results concerning entire set of proteins were generally as expected on the basis of data from tropomyosins annotated in BIOPEP database [Minkiewicz *et al*., 2011]. Epitopes from shrimp tropomyosin were present in 484 proteins. Tropomyosins (i.e. proteins possessing the tropomyosin domain with the signature IPR000533 in the InterPro database) were the most abundant family (Table 3). 331 tropomyosins contained fragments identical to those of the Pen a 1.0102 allergen. Among these sequences, 192 were annotated in Allergome database, which is the leading to date database of allergens. Most invertebrate species may be divided into five categories on the basis of kind of contact with people (Table 4). The first of them summarises edible invertebrates – mainly crustaceans and molluscs (61 species). The second one contains human parasites – worms and arthropods, *e.g.* blood-feeding (24 species). The third one includes parasites of domestic and/or edible animals (19 species). The fourth category includes parasites of edible plants (7 species). The fifth category includes invertebrates having contact with humans, such as dust mites, insects and other arthropods possible to be found in houses (18 species). Cultured invertebrates such as *Bombyx mori* (economically important), *Drosophila melanogaster* or *Caenorhabditis elegans* (cultured in research laboratories) are also included in this category. Some parasites are classified in two categories (human and animal parasites) and they possess more than one host.

The broad prevalence of common epitopes among tropomyosins from various sources is in agreement with experi-

TABLE 3. Protein families containing epitopic peptides identical to those from allergen Pen a 1.0102, annotated in the InterPro database. Only families containing at least two proteins with the above-mentioned epitopes are included.

No	Signature of domain, family or superfamily	Name of domain, family or superfamily	Number of proteins containing epitopes	Percentage of proteins containing epitopes (%)
1.	IPR000032	Phosphotransferase system, phosphocarrier HPr protein-like	5	0.048
2.	IPR000182	GNAT domain	2	0.001
3.	IPR000533	Tropomyosin	331	21.564
4.	IPR000536	Nuclear hormone receptor, ligand-binding, core	3	0.039
5.	IPR001020	Phosphotransferase system, HPr histidine phosphorylation site	5	0.063
6.	IPR001209	Ribosomal protein S14	4	0.049
7.	IPR001519	Ferritin	5	0.101
8.	IPR001623	Heat shock protein DnaJ, N-terminal	2	0.006
9.	IPR002114	Phosphotransferase system, HPr serine phosphorylation site	5	0.091
10.	IPR002544	FMRFamide-related peptide-like	2	1.026
11.	IPR003742	SPOUT methyltransferase, predicted	2	0.043
12.	IPR004640	Co-chaperone Hsc20	2	0.101
13.	IPR005698	Phosphotransferase system, phosphocarrier HPr protein	5	0.048
14.	IPR005746	Thioredoxin	2	0.009
15.	IPR006450	Bacteriophage HK022, Gp6	2	0.219
16.	IPR006524	Transcription activator, ArpU family	4	0.435
17.	IPR007214	YbaK/aminoacyl-tRNA synthetase-associated domain	5	0.040
18.	IPR008331	Ferritin/DPS protein domain	5	0.038
19.	IPR008946	Nuclear hormone receptor, ligand-binding	4	0.046
20.	IPR009040	Ferritin – like diiron domain	5	0.049
21.	IPR009073	Heat shock cognate protein B, C-terminal oligomerisation	2	0.070
22.	IPR009078	Ferritin/ribonucleotide reductase-like	5	0.013
23.	IPR010360	Protein of unknown function DUF956	2	0.223
24.	IPR011991	Winged helix-turn-helix transcription repressor DNA-binding	2	0.0003
25.	IPR012336	Thioredoxin-like fold	2	0.001
26.	IPR012347	Ferritin-related	5	0.017
27.	IPR013766	Thioredoxin domain	2	0.008
28.	IPR016051	Ribosomal RNA large subunit methyltransferase H	2	0.066
29.	IPR016181	Acyl-CoA N-acyltransferase	2	0.001
30.	IPR018271	Ribosomal protein S14, conserved site	3	0.051
31.	IPR020994	Uncharacterised protein family, calcium binding protein, CcbP	2	3.704
32.	IPR021146	Bacteriophage QLRG family, putative DNA packaging	2	0.083
33.	IPR023036	Ribosomal protein S14, bacterial/plastid	4	0.089
34.	IPR024320	Lysylphosphatidylglycerol synthetase, domain of unknown function DUF2156	2	0.066
35.	IPR024344	Mycothiol-dependent maleylpyruvate isomerase, metal-binding domain	2	0.078
	Not attributed		68	

TABLE 4. Selected species synthesising tropomyosins containing epitopic peptides identical to those from *Farfantepenaeus aztecus* tropomyosin.

Category	Species
Edible invertebrates	*Anadara broughtonii; Argopecten irradians; Artemia franciscana; Balanus rostratus; Charybdis feriatus; Chionoecetes opilio; Chlamys nipponensis akazara; Crangon crangon; Crassostrea gigas; Crassostrea virginica; Erimacrus isenbeckii; Eriocheir sinensis; Euphausia pacifica; Euphausia superba; Farfantepenaeus aztecus; Fenneropenaeus chinensis; Fenneropenaeus merguiensis; Fulvia mutica; Haliotis asinina; Haliotis discus discus; Haliotis diversicolor; Haliotis rufescens; Helix aspersa; Homarus americanus; Jasus lalandii; Limulus polyphemus; Litopenaeus vannamei; Loligo bleekeri; Macrobrachium rosenbergii; Metapenaeus ensis; Mimachlamys nobilis; Mizuhopecten yessoensis; Mytilus edulis; Mytilus galloprovincialis; Neptunea polycostata; Octopus vulgaris; Ommastrephes bartramii; Oratosquilla oratoria; Pandalus borealis; Pandalus eous; Paralithodes camtschaticus; Penaeus japonicus; Penaeus monodon; Perna viridis; Portunus sanguinolentus; Portunus trituberculatus; Procambarus clarkii; Scylla olivacea; Scylla serrata; Sepia esculenta; Sepia officinalis; Sepioteuthis lessoniana; Sinonovacula constricta; Solen strictus; Spisula sachalinensis; Squilla aculeate; Squilla oratoria; Todarodes pacificus; Tresus keenae; Turbo cornutus; Venerupis philippinarum*
Human parasites	*Aedes aegypti; Amblyomma maculatum; Amblyomma variegatum; Anopheles darlingi; Anopheles gambiae; Ascaris lumbricoides; Brugia malayi; Clonorchis sinensis; Culex quinquefasciatus; Dermanyssus gallinae; Echinococcus granulosus; Echinococcus multilocularis; Glossina morsitans morsitans; Haemaphysalis longicornis; Haemaphysalis qinghaiensis; Ixodes scapularis; Onchocerca volvulus; Pediculus humanus subsp. corporis; Schistosoma haematobium; Schistosoma japonicum; Schistosoma masoni; Trichinella pseudospiralis; Trichinella spiralis; Trichostrongylus colubriformis*
Parasites of domestic and/or edible animals	*Amblyomma maculatum; Angiostrongylus vasorum; Anisakis simplex; Ascaris suum; Boophilus microplus (Rhipicephalus microplus); Caligus clemensi; Caligus rogercresseyi; Clonorchis sinensis; Dermanyssus gallinae* and other species from *Dermanyssus* genus*; Haemaphysalis longicornis; Haemaphysalis qinghaiensis; Lepeophtheirus salmonis; Onchocerca ochengi; Ornithonyssus sylviarum; Psoroptes ovis; Schistosoma turkestanicum; Teladorsagia circumcincta; Trichinella pseudospiralis*
Parasites of edible plants	*Acyrthosiphon pisum; Heligmosomoides polygyrus; Heterodera glycines; Maconellicoccus hirsutus; Myzus persicae; Radopholus similis; Toxoptera citricida*
Other invertebrate species possessing contact with humans	*Acarus siro; Aleuroglyphus ovatus; Blattella germanica; Blomia tropicalis; Bombyx mori; Caenorhabditis elegans; Camponotus floridanus; Chironomus kiiensis; Dermatophagoides farinae; Dermatophagoides pteronyssinus; Drosophila melanogaster; Glycyphagus domesticus; Lepidoglyphus destructor; Lepisma saccharina; Periplaneta americana; Periplaneta fuliginosa; Tribolium castaneum; Tyrophagus putrescentiae*

mental results concerning cross-reactivity, reviewed by Bessot and *et al.* [2010] as well as Caraballo & Acevedo [2011].

Six proteins, not belonging to tropomyosin family contain epitopic peptides consisting of 8 residues (Table 5).

Occurrence of the rare pentapeptides

Rare pentapeptide DEERM (IEDB ID 7975) is the most abundant common epitope between Pen a 1.0102 and proteins not belonging to the tropomyosin family. Rare pentapeptides are considered as basic motifs responsible for stimulation of the immune system [Kanduc, 2008, 2012]. Pentapeptide may be considered as rare if it only occurs in hundreds of protein sequences. According to the database associated with the Tachyon program [Tan *et al*., 2012], the most abundant pentapeptides occur in three orders of magnitude higher number of sequences.

Pentapeptide DEERM from shrimp tropomyosin was present in 147 proteins not belonging to tropomyosin family, *i.e.* not attributed or containing domains other than tropomyosin. Well-defined domains, annotated in the InterPro system were present in 85 protein sequences. Proteins contained 123 domains annotated in the InterPro system, but only 35 of them were present in two or more proteins (Table 3). Apart from tropomyosin, no domain was present in more than five proteins. Only in two cases (FMRFamide-related peptide-like (IPR002544) and uncharacterised protein family, calcium binding protein CcbP (IPR020994) did the percentage of proteins with epitopes from Pen a 1.0102 exceed 1% of proteins containing a domain. Selected species synthesising proteins containing pentapeptide DEERM are organised into categories as described previously [Minkiewicz *et al*., 2012] (Table 6): edible plants and animals (7 species), microorganisms involved or potentially involved in food technology (2 species), human symbionts and commensals (12 species) as well as human pathogens (18 species). Some species are annotated in two categories: human symbionts and commensals as well as human pathogens. They are microorganisms usually coexisting with humans but sometimes causing opportunistic infections.

Particular families contain up to five proteins with the epitopes (Table 3) and usually between 0.01 and 0.1% of proteins within a family containing epitopes. The distribution of the peptide DEERM, among protein families (apart from tropomyosins) seems to be random. This finding is consistent with the distribution of hexapeptides from *Gadus morhua* subsp. *callarias* parvalbumin [Minkiewicz *et al*., 2012]. The fact that pathogens are the most abundant category among the species synthesising proteins not belonging to the tropomyosin family and containing epitopes from Pen a 1.0102 is also in line with previous findings [Minkiewicz *et al*., 2012]. The presence of the same epitopes in allergens and pathogens should be taken into account during vaccine design, as discussed previously [Minkiewicz *et al*., 2012]. The epitope with sequence DEERM occurs *e.g.* in proteins from human gastrointestinal microflora. The abnormal interactions between these microorganisms and human immune system are associated with the Inflammatory Bowel Disease [Glocker & Grimbacher, 2012]. The above mentioned epitope has been found also in three human protein sequences, charged multivesicular body proteins 7: E5RFR8, E5RJI3

TABLE 5. Proteins non-homologous with *Farfantepenaeus aztecus* tropomyosin, containing fragments longer than 5 amino acid residues, identical to epitopes of the above-mentioned protein.

Epitope sequence	Proteins*
SDEERMDA [Reese *et al*., 2005]	TR:F5SP91_9GAMM; TR:D4J5G4_9FIRM; TR:E5A7V5_LEPMJ
LENQLKEA (IEDB ID 35633)	TR:F3ZNR4_9BACE; TR:D2W6T4_NAEGR; TR:B6AGL9_CRYMR

* Entry names in UniProt database are given.

TABLE 6. Selected species containing proteins non-homologous with *Farfantepenaeus aztecus* tropomyosin, containing epitopes identical to those of the above-mentioned protein.

Category	Species
Edible plants and animals	*Acipenser transmontanus; Camellia sinensis; Gallus gallus; Laccaria bicolor; Oryza sativa* subsp. *Japonica; Sorghum bicolor; Vitis vinifera*
Microorganisms involved or potentially involved in food technology	*Debaryomyces hansenii; Lactobacillus crispatus*
Human symbionts and commensals	*Bacteroides coprocola; Bacteroides intestinalis; Coprococcus catus; Corynebacterium amycolatum; Enhydrobacter aerosaccus; Eubacterium cylindroides; Holdemania filiformis; Jonquetella anthropic; Lactobacillus crispatus; Marvinbryantia formatexigens; Pediococcus acidilactici; Streptococcus sanguinis*
Human pathogens and parasites	*Ajellomyces dermatitidis; Asticcacaulis biprosthecum; Bacteroides ovatus; Clostridium difficile; Coccidioides posadasii; Comamonas testosterone; Corynebacterium amycolatum; Corynebacterium jeikeium; Cryptosporidium muris; Enterococcus faecium; Jonquetella anthropic; Kocuria rhizophila; Legionella pneumophila; Parachlamydia acanthamoebae; Porphyromonas endodontalis; Streptococcus sanguinis; Streptococcus sp.; Weeksella virosa*

and B4DKJ6. They are not attributed to any family defined in InterPro classification system.

The fact that pathogens are the most abundant category among the species synthesising proteins not belonging to the tropomyosin family and containing epitopes from Pen a 1.0102 is also in line with previous findings [Minkiewicz *et al*., 2012]. The similarity between proteins is also molecular basis of so called hygiene hypothesis assuming that in the case of lack of pathogens, the immune system may be provoked by proteins similar to those originated from pathogens [da Costa Santiago *et al*., 2013].

Pentapeptides as fragments of epitopes interacting with IgE and originating from various allergens have previously been found in human protein sequences [Kanduc, 2008]. The fragments of tropomyosin from *Farfantepenaeus aztecus* were among them. Human tropomyosin is considered as an autoantigen in inflammatory bowel disease [Mirza *et al*., 2006].

Advantages and limitations of prediction based on common subsequences

The major advantage of the database screening using epitope sequences is simplicity and possibility of rapid construction of preliminary list of potential cross-reacting allergens. Results of such screening may serve as the basis for further research. Presence of common fragments recognised as epitopes seems to be a stronger criterion to taking into attention possibility of cross-reaction than criterion assuming presence of any common fragment containing at least 6–8 amino acid residues. "Stronger" may be understood as leading to smaller percentage of false positive results. Suitability of presence of common epitopes increases if allergenicity is triggered by fragments of proteins *e.g.* originating from proteolysis occurring during food processing or digestion. The presence of common sequential epitopes as a molecular basis of cross-reactivity between tropomyosins has some experimental support. Mouse tropomyosin with inserted epitopes from *Farfantepenaeus aztecus* tropomyosin revealed interaction with immunoglobulin E [Albrecht *et al*., 2009], *i.e.* fulfilled one of the three criteria of allergenicity.

Some limitations of database screening using epitope sequences as queries have been discussed previously [Minkiewicz *et al*., 2011]. In the above publication we have pointed out that searching for protein fragments identical with query sequence leads to miss some allergens. In the case of tropomyosins the Ore m 4.0101 allergen may serve as an example of false negative result. Likelihood of obtaining false negative results may be lowered if similarity between query sequence and fragment of target sequence is below 100%. It is obvious that cross-reactivity based on conformational epitopes is also missed.

Epitope mapping is usually performed using synthetic fragments of protein chain or to a lesser extent fragments obtained *via* proteolysis. Results obtained using peptides not always may be extrapolated to the entire proteins. Part of peptides may not fulfil recommendation concerning the secondary structure consistent with the secondary structure of parent proteins.

In our work we have used a set of 60 peptide sequences as queries. For 40 of them α-helix was pointed out as the most likely secondary structure for more than 50% of chain length. The most likely sequence (for at least 50% of chain) for the remaining 20 peptides was coil. It is possible that peptide without defined structure will interact with IgE whereas entire proteins containing the fragment with identical sequence but

possessing α-helix structure – will not. On the other hand, coiled peptides may serve for prediction of allergenicity of denatured or partially hydrolysed proteins. Denaturation or partial hydrolysis often occurs during food processing. Protein fragments may also lose secondary structure during digestion in the digestive tract.

In the case of rare pentapeptides, such as DEERM, more complex analysis of their surrounding may be necessary. This peptide is too short to form well-defined secondary structure. When peptide is a fragment of longer chain it may form α-helix or coil depending on surrounding. Apart from secondary structure the location of a fragment at protein surface, charge and hydrophobicity fragment surrounding should be included.

CONCLUSIONS

The work presented describes the first survey of prevalence of epitopes from Pen a 1.0102 allergen in universal proteome.

Vertebrate tropomyosins (*e.g.* from vertebrates used as food resources) contain fragments containing between 10 and 15 amino acid residues revealing 100% identity with epitopes from allergen Pen a 1.0102 (tropomyosin from shrimp *Farfantepenaeus aztecus*). Fragments identical to epitopes from Pen a 1.0102 are common in sequences of invertebrate tropomyosins, including these annotated in the Allergome database. Common epitopes are a likely molecular basis for cross-reactivity between them (*e.g.* between food and non-food invertebrates). Some epitopes, especially rare pentapeptides (with the DEERM sequence), are present in sequences of proteins not sharing homology with tropomyosins. This fragment is present in several proteins, *e.g.* from edible plants and animals as well as pathogenic microorganisms.

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