Identification and Characterization of Tropomyosin in a Potential Shrimp OIT Product

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Abstract

Rationale: Shrimp allergy is the second most common food allergy in the U.S.A., affecting up to 1.2% of the pediatric population. Oral immunotherapy (OIT) to food allergy decreases the threshold of clinical reactivity to the specific food. Given the risk of clinical reactions to shrimp allergen with accidental exposures, shrimp OIT may be an effective treatment. Identification of the appropriate OIT product, major shrimp allergen, tropomyosin (Pen a 1) is critical.
Methods: Identification and characterization in a shrimp OIT product of major shrimp allergen tropomyosin (Pen a 1), compared to placebo, oat. Evaluation was made by SDS-PAGE and mass spectrometry analysis performed with digested peptides from utilizing a nanoHPLC-Q Exactive Mass Spectrometer. The proteome Discoverer 1.4 interface with Mascot algorithm was utilized to identify recovered peptides. Immunological analysis was made by western blot with anti-shrimp tropomyosin.
Results: SDS-PAGE gel identified shrimp tropomyosin at 37kD in the OIT shrimp but not in the oat product. The tropomyosin database was generated from NCBI database by selecting proteins which have tropomyosin in protein description regardless of taxa (283 entries). In the oat sample, two shrimp tropomyosin peptides were identified. The data indicated that based on the area under the curve oat has 0.01% of tropomyosin concentration compared to shrimp. Recovered tropomyosin peptides from oat shares may be due to contamination of insects around oat grains. Western blot analysis indicated exclusive signal of tropomyosin from the shrimp sample.
Conclusion: Identification and quantification of Pen a 1 from a potential shrimp OIT product resulted in the confirmation of this product to be a potential candidate for OIT in shrimp allergy.

Introduction

Shrimp allergy is the second most common food allergy in the United States, affecting up to 1.3% of the pediatric population, disproportionately increased in those with asthma, and considered a lifelong disease. Accidental exposure to shrimp in allergic patients is a major cause of visits to the emergency room, with life threatening anaphylaxis occurring in up to 50% of those shellfish allergic. Childhood shrimp allergy is a serious and under-diagnosed problem. There is no cure for shrimp allergy and this is a critical need to develop an effective treatment for shrimp allergic patients. In the absence of such treatment, millions of patients will continue to be at risk for life threatening anaphylaxis and treatment options will remain limited.
Shrimp allergens are cross reactive with allergens from house dust mites (HDM) and cockroach, with up to 97.5% homology, and tropomyosin sensitization is found in both HDM and cockroach allergic patients. The role of this sensitization is currently controversial with evidence that HDM allergy immunotherapy is protective against shrimp allergy as well as data suggestive that HDM allergy increases sensitization. Oral HDM ingestion in infants has been associated with a decrease in overall sensitization. However, the role of HDM and cockroach allergies have never been evaluated within the context of OIT to determine the role of aeroallergen exposure in the transfer from a Th2 to a Th1 immunologic response in shrimp allergic patients. Given the extensive cross reactivity of shrimp with other arthropods containing the major allergen tropomyosin, this proposed clinical trial would allow an evaluation of the role of sensitization to cockroach and HDM on the expression of shrimp allergy and outcomes of OIT.

Methods

The drug substance is shrimp flour. Tropomyosin (Pen a 1), a protein from muscle, has been the first shrimp allergen detected. It is a major allergen of 38-41 kDa, and it is responsible for cross-reactivity between members of the shellfish family, particularly among the crustaceans. This led to its definition as an invertebrate panallergen. Different regions of shrimp tropomyosin bind IgE; 5 major IgE-binding regions have been identified in shrimp tropomyosin containing at least 8 epitopes and 28 CD4+T cell epitopes.

Item Description/Name	Manufacturer	Cat#	Grade	Acceptance Criteria
Shrimp, *Litopenaeus/Farfantepenaues* Shrimp flour	Stallergenes Greer	RMF34P	Food	COA

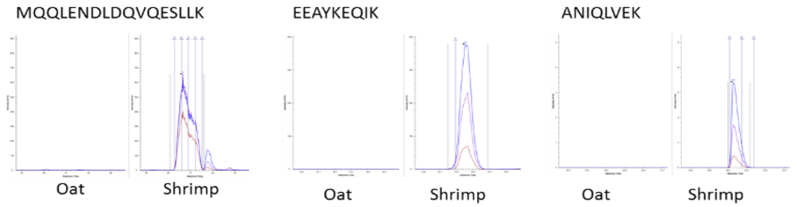
Detection of Shrimp tropomyosin from shrimp and oat samples
Baylor College of Medicine Pathway Discovery Mass Spectrometry/ Identification of Protein Complexes Core Facility (BCM-PDMSC):

Protein Digestion and Mass Spectrometry Analysis:

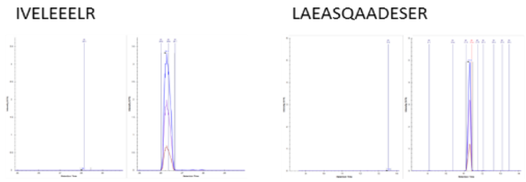
- 30 ug of protein extract was incubated with 1.5 ug of sequencing grade trypsin for overnight and on next day another 0.3 ug of trypsin added and incubated for 4hrs.
- 1 ug of digested peptide was analyzed using nanoHPLC-Q Exactive Mass Spectrometry instrument (Thermo Scientific)
- Proteome Discoverer 1.4 interface (Thermo Fisher) with Mascot algorithm (Mascot 2.4, Matrix Science) was used to identified recovered peptide using target-decoy tropomyosin database. The tropomyosin database was generated from NCBI nr database by selecting proteins which has tropomyosin in protein description regardless taxa (Containing 283 entries).
- Western Blot:
- Immunological analysis was made by western blot analysis with an anti-shrimp tropomyosin (Indoor Biotechnologies). As shown in the western blot, shrimp tropomyosin was identified at 37kD

Mass Spectrometry of Tropomyosin

A) Examples of tropomyosin peaks exclusively detected from Shrimp sample



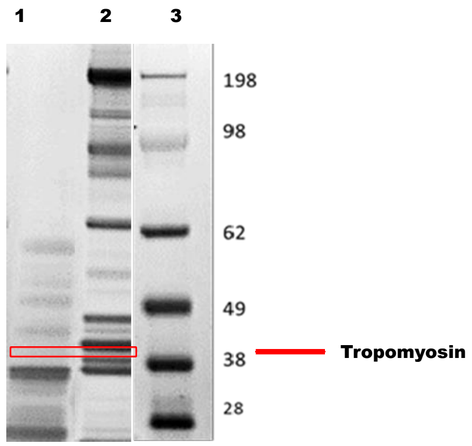
B) Two of tropomyosin peptides detected from both of oat and shrimp samples



Peptide Sequence	Peptide Peak area	
	Oats	Shrimp
IVELEEELR	7.14E+07	1.15E+11
LAEASQAADESER	5.03E+07	9.77E+10

Recovered tropomyosin peptides from oat share homology with tropomyosin from various species, not only for shrimp. Which indicate that the existence shrimp tropomyosin in oat sample may due to contamination of insect to oat grains.

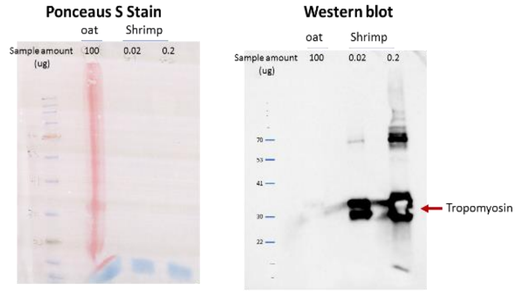
SDS-PAGE Anaylsis of Oat and Shrimp



1. Oat
2. Shrimp (Stallergenes Greer)
3. MW Ladder

SDS-PAGE gel (NuPAGE Bis Tris 4%-12% gradient gels (Invitrogen)), shrimp tropomyosin was identified at 37kD in the shrimp flour (Stallergenes Greer) but was not evident in the, placebo, oat flour (GF Harvest)

Western Blot Shrimp Tropomyosin



Primary Ab: Rabbit anti Shrimp Tropomyosin PA-SHM, Vendor: Indoor biotechnologies (Cat#: EL-TPM) Dilution 1: 50k

Secondary Ab: HRP-conjugated Goat Anti-Rabbit IgG (H+L) Vendor: Jackson ImmunoResearch Laboratories, Inc (Cat#: 111-035-144) Dilution: 1:10k

Western blot result shows exclusive signal of tropomyosin from shrimp sample only. The shrimp sample shows signal with 0.02 % protein compared to oat sample amount. The result indicate that oat has no detectable tropomyosin from tested amount

List and Area-Under-Curve of Shrimp Tropomysin Peptides Detected from Shrimp and Oat Samples

Peptide Sequence	Peptide Peak area	
	Oats	Shrimp
IVELEEELR	7.14E+07	1.15E+11
LAEASQAADESER	5.03E+07	9.77E+10
ADTLEQQNK	n.d.	5.49E+07
AEKSEEEVHNLQK	n.d.	1.73E+09
ALSNAEGEVAALNR	n.d.	1.64E+11
ANIQLVEK	n.d.	7.31E+10
ANIQLVEKDK	n.d.	2.39E+09
DKALSNAEGEVAALNR	n.d.	7.21E+08
EEAYKEQIK	n.d.	8.51E+09
EVDRLEDELVNEK	n.d.	1.27E+11
EVDRLEDELVNEKEK	n.d.	2.08E+10
FLAEEADR	n.d.	6.38E+10
KLAMVEADLER	n.d.	2.03E+09
LAMVEADLER	n.d.	6.58E+10
LEDELVNEK	n.d.	2.59E+10
MDALENQLK	n.d.	7.46E+10
MQQLENDLDQVQESLLK	n.d.	1.21E+11
SEEEVHNLQK	n.d.	6.82E+08
SITDELDQTFSELSGY	n.d.	2.49E+08
SLEVSEEK	n.d.	6.26E+10
SLEVSEEKANQR	n.d.	1.08E+08
SLSDEER	n.d.	4.10E+07
YDEVAR	n.d.	4.76E+07
Total Peptide area	**1.22E+08**	**1.03E+12**

In the oat sample we identified two shrimp tropomyosin peptides, but the peptide AUC (area under the curve, relative protein amount) shows the amount is drastically different. Based on AUC oat has 0.01% of tropomyosin concentration compare to shrimp.

Conclusion

Identification and quantitation of Pen a 1 was made in shrimp powder with successful identification of tropomyosin (pen a 1) to be different from oat to be utilized in shrimp OIT. Given the risk of clinical reactions to shrimp allergen with accidental unintentional exposures, shrimp OIT may be an effective treatment that would fill an unmet need. Subjects receiving this therapy need close monitoring through expert centers to treat potential systemic symptoms. The immune determinants of clinical tolerance are still not completely understood.