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Molecular and immunological approaches in quantifying the air-borne food allergen tropomyosin in crab processing facilities

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ABSTRACT

Tropomyosin is a cross-reactive allergenic protein present in ingested shellfish species. Exposure and sensitization to this protein via inhalation is particularly important in the crustacean processing industry where workers are continuously exposed to the aerosolized form of this allergen. The aim of this study was to develop an antibody-based immunoassay to enable the specific and sensitive quantification of aerosolized tropomyosin present in the environment of two crab processing facilities.

Anti-tropomyosin antibody was generated in rabbits against tropomyosins from four different crustacean species. These antibodies were purified using recombinant tropomyosin using an immuno-affinity column. The recombinant tropomyosin was also used as an allergen standard for the sandwich ELISA. In order to quantify aerosolized tropomyosin, air collection was performed in the personal breathing zone of 80 workers during two crab processing activities, edible crab (*Cancer pagurus*) and king crab (*Paralithodes camtschaticus*) using polytetrafluoroethylene filters. The purified antibody was able to detect tropomyosin selectively from different crustaceans but not from vertebrate sources. The limit of detection (LOD) for the developed sandwich ELISA was 60 picogram/m^3 and limit of quantitation (LOQ) 100 picogram/m^3. Immunoassay validation was based on linearity (R^2 0.999), matrix interference test (78.8 ± 6.5%), intra-assay CV (9.8%) and inter-assay CV (11%). The novel immunoassay was able to successfully identify working activities, which generated low, medium or high concentrations of the aerosolized food allergen.

We describe an IgG antibody-based immunoassay for quantification of the major food allergen tropomyosin, with high sensitivity and specificity. This modified immunological approach can be adapted for the detection of other aerosolized food allergens, assisting in the identification of high-risk allergen exposure areas in the food industry.

Introduction

Occupational allergy and asthma has become a serious health concern, especially for workers in the seafood industry. Increased global consumption and changing dietary habits have greatly facilitated seafood production (Lopata and Jeebhay, 2013; Lopata and Lehrer, 2009). This in turn, has caused more workers to be exposed to seafood allergens on a daily basis. According to a report by the Food and Agriculture Organization (FAO) in 2010, nearly 45 million people are involved in seafood and aquaculture production. Several studies have shown that the prevalence of occupational asthma among workers exposed to shellfish is between 4% and 36% (Bonlokke et al., 2012; Granslo et al., 2009; Howse et al., 2006). Moreover, workers with occupational asthma to shellfish

Abbreviations: Tm, tropomyosin; rTm, recombinant tropomyosin; cAb-αTM, anti-tropomyosin capture antibody; dAb-αTM, detection antibody.

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were shown to develop ingestion-related food allergies to the same shellfish species (Jeebhay et al., 2001). Occupational exposure to shellfish allergens can elicit upper and lower respiratory tract symptoms such as asthma, rhinitis and can even cause skin symptoms (Aasmoe et al., 2005; Bang et al., 2005; Lopata and Jeebhay, 2013).

In the seafood industry, workers are constantly exposed to air-borne shellfish particulate matter arising from the different processing techniques. Several studies have shown the presence of allergenic proteins in air-borne particulate matter. These allergens are responsible for causing allergic sensitization among affected workers (Abdel Rahman et al., 2011, 2013; Taylor et al., 2000). Serum IgE antibody reactivity to crab proteins among snow crab processing workers due to occupational exposure of crab matter has been reported previously (Cartier et al., 1984, 1986; Gill et al., 2009; Malo et al., 1997; Weytjens et al., 1999). Aerosolization of shellfish allergens occurs due to processes such as filleting, freezing, cooking, smoking, drying and techniques using high pressure water or air (Jeebhay et al., 2001; Lopata and Jeebhay, 2013). Processes such as butchering, de-gilling and particularly boiling, have been shown to cause excessive bioaerosol formation. The wet or dry air-borne particles may then be inhaled by exposed workers. Abdel Rahman et al. demonstrated elevated levels of air-borne crab allergens in specific work stations such as butchering and cooking as compared to cleaning, packing and storage (Abdel Rahman et al., 2012).

The commonly consumed shellfish can be divided in two groups; crustaceans (shrimps, crabs, lobsters) and mollusks (oyster, mussels, octopus, squid) (Lopata and Kamath, 2012; Lopata et al., 2010). The major shellfish allergen is a 33–36 kDa muscle protein called tropomyosin (TM). Over 80% of shellfish sensitized patients are known to react to this major allergen. As a muscle protein, tropomyosin exists in a dimeric confirmation which complexes with troponin to cover the actin-binding sites during muscle contraction. Due to this role, tropomyosin is a highly expressed protein found mainly in the edible meat. Tropomyosin displays a remarkable stability to heating and is able to retain its allergenicity even in heat-processed shellfish products (Abramovitch et al., 2013; Johnston et al., 2014; Kamath et al., 2013).

Tropomyosin displays high amino acid sequence (primary structure) identity within crustaceans, ranging from 95% to 100% (Kamath et al., 2013). Interestingly, crustacean tropomyosin shares a certain degree of amino acid homology of 75–83% sequence identity with house dust-mite and insect tropomyosins. A variety of mite and cockroach allergens have been implicated in air-borne exposure and sensitization, however, tropomyosin is only a minor allergen in both allergens sources. This is most likely due to low immunological cross-reactivity as well as low relative abundance of this protein compared to consumed shellfish (Arlian et al., 2009; Pomes et al., 2007).

Several studies have shown that antibody reactivity to tropomyosin is a good predictor of shellfish allergy (Gámez et al., 2011; Kamath et al., 2013). Due to its excellent structural stability and detailed characterization, tropomyosin was chosen as an ideal molecular marker for detecting air-borne shellfish allergens in this study.

We report the development and validation of a highly sensitive immunoassay to detect and quantify aerosolized tropomyosin in air samples collected from crab processing factories. Using this immunoassay, we were able to quantify air-borne tropomyosin in a worker- and activity-specific manner.

The approach of using a recombinant protein as standard and purified natural allergen to generate the capture antibody for increased sensitivity and specificity has not been employed previously.

This methodology can be modified for the quantification of other major food allergens and would be an important tool in monitoring air-borne allergen levels in different work environments. This can subsequently assist in establishing safety paradigms to control the unintentional generation of aerosolized allergens and accidental sensitization of exposed workers.

Methods

Allergen standard: expression and purification of recombinant tropomyosin

Recombinant tropomyosin was expressed and purified as described previously (Kamath et al., 2013). Briefly, total RNA was extracted from fresh specimens of black tiger prawn (*Penaeus monodon*) using the RNAeasy mini extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Complementary DNA (cDNA) was reverse transcribed from the total RNA using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Basel, Switzerland), following the manufacturer's instructions. The coding region for tropomyosin was amplified by PCR using forward 5′-GCGGATCCGACGCCATCAAGAAGAAGATGC-3′ and reverse 5′-GCGAATTCTTAGTAGCCAGACAGTTCGCTG-3′ primers. The PCR conditions were set as follows, 94 °C for 2 min, 30 cycles of 94 °C for 20 s, 55 °C for 20 s, 72 °C for 30 s and a final elongation step, 72 °C for 7 min. The 860 bp amplified product was cloned into the expression vector pRSET-A using the *BamH1* and *EcoR1* restriction sites. The recombinant expression vector, pRSET-A-TM was transformed into BL21 *Escherichia coli* strain and expression of the recombinant tropomyosin with a HIS-tag, induced using 1 mM Isopropyl β-D-1-thiogalactopyranoside (Amresco, USA). The bacterial cells were washed with extraction buffer (25 mM Tris–HCl, pH 8.0, 300 mM NaCl, 1 mM imidazole) and lysed using a French pressure cell. After centrifugation at 6000 × *g* for 15 min, the recombinant tropomyosin was purified using HIS-Trap FF Affinity Column (GE Healthcare, USA). The fraction containing the recombinant protein was further purified using a Superdex G75 16/600 size exclusion column (GE Healthcare, USA) on a Biologic Duoflow FPLC (BioRad, Hercules, CA, USA). The purified recombinant tropomyosin was labeled "rTm" and stored in aliquots at −80 °C until further use.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed as described earlier (Abdel Rahman et al., 2010) to confirm the purity of the tropomyosin standard and analyze the binding characteristics of the purified antibodies.

Twelve micrograms of the protein samples was heated in Laemmli buffer containing dithiothreitol and loaded onto a 12% bis-acrylamide gel. Protein separation was performed at 180 V using a Mini-Protean Tetra Cell electrophoresis system (BioRad, Hercules, CA, USA). The separated proteins were visualized by staining with Coomassie brilliant blue R250 (BioRad, Hercules, CA, USA).

CD spectroscopy of allergen standard

Circular dichroism spectroscopy was performed to analyze the alpha helical content of rTM and compare it to purified natural prawn tropomyosin. Natural and recombinant tropomyosin samples were prepared in PBS, pH 7.2 and adjusted to a final concentration of 3 μM. CD spectroscopy was performed on a J715 Spectropolarimeter (Jasco, USA) with continuous nitrogen flushing at 25 °C. All measurements were performed using a 10 mm quartz cuvette over a wavelength range of 190–260 nm. For wavelength analysis, the tropomyosin samples were scanned with a step width of 0.2 nm and bandwidth of 1 nm at 100 nm/min averaging over