

Myosin Heavy Chain: An Allergen Involved in Anaphylaxis to Shrimp Head

Saenz de San Pedro B¹, López Guerrero A¹, Navarrete del Pino MA¹, Alcántara Villar M¹, Álvarez F², López-Matas MA², Carnés J²

¹Allergology Department, Hospital Universitario Jaén, Spain

²R&D Unit, LETI Pharma S.L.U., Tres Cantos, Madrid, Spain

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Seafood is one of the most prevalent sources of food allergens and is becoming a leading cause of anaphylaxis worldwide [1-3]. Nine shrimp allergens have been reported to date (www.allergen.org). Allergy to shrimp is generally diagnosed using preparations based on shrimp body. This could account for misdiagnoses or even false-negative results in patients who consume shrimp head. In this study, we investigated a patient with allergic symptoms after consumption of shrimp head and identified a myosin heavy chain (MHC) as the culprit allergen.

The patient was a 30-year-old man who had experienced multiple episodes of pruritic generalized rash, dyspnea, and dysphagia approximately 7-8 hours after ingestion of crustaceans. In the most recent episode, after ingestion of shrimp, he required emergency care, although he responded well to intravenous medication. He reported 1 episode of fecal incontinence with loss of consciousness. No cofactors were associated with the reaction. He occasionally tolerates crustaceans and has good tolerance to mollusks and fish.

Skin prick testing was positive with *Anisakis* (Roxall Group) (5 mm), and prick-by-prick testing was positive with shrimp head (8 mm) and crayfish head (5 mm) and negative with shrimp and crayfish body. Testing of shrimp head and body was performed separately because the patient usually sucks the crustacean heads. Total IgE was 113 kU/L; specific IgE was 11 kU/L for *Anisakis* and 0.48 kU/L for shrimp and negative for tropomyosin and mites (*Dermatophagoides pteronyssinus* and *Lepidoglyphus destructor*) (ImmunoCAP, Thermo Fisher Scientific).

Shrimp (*Penaeus* species) was purchased at a local market and used to prepare head and body extracts. The content of the heads was removed with a spatula and the bodies were peeled. Afterwards, both were boiled for 10 minutes in phosphate-buffered saline 0.01 M and saline solution (NaCl) 0.15 M, homogenized, and shaken at 4°C for 4 hours. Finally, head and body extracts were centrifuged, dialyzed, sterile filtered, and lyophilized. The protein content per milligram of lyophilized extract was analyzed using the Bradford method (Thermo

Fisher Scientific), yielding results of 63.1 µg and 576.7 µg for head and body, respectively.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was used to analyze the protein profile of both extracts. Twenty micrograms of protein from each extract was loaded in the gel and run under reducing conditions. The molecular weights ranged from 10 to 40 kDa for head and from 10 to 150 kDa for body (Figure, B).

The protein bands recognized by the IgE from the patient's serum were determined by immunoblotting. Proteins separated by SDS-PAGE were transferred to a Trans-Blot Turbo PVDF membrane (Bio-Rad) and incubated overnight with the patient's serum diluted 1/2. Horse radish peroxidase–conjugated mouse antihuman IgE (Southern Biotech) was used as secondary antibody, and ECL Prime Western Blotting Detection (Amersham) was used to detect the allergenic proteins by means of chemiluminescence. A single band was detected at approximately 40 kDa in the shrimp head, and no bands were detected in the shrimp body (Figure, D). Cross-reactivity between shrimp body proteins and the 40-kDa protein recognized in shrimp head was ruled out by immunoblot inhibition (data not shown).

Additionally, 2-dimensional electrophoresis (2D) was used to investigate the protein profile in more detail. Shrimp head extract was washed using the ReadyPrep 2D Cleanup Kit (Bio-Rad) and the proteins were separated according to their isoelectric point in ReadyStrip IPG (Bio-Rad) at a pH range of 3-10. After the first dimension, the proteins were separated in the second dimension according to their molecular weight. Gel was stained with Oriole Fluorescent Gel Stain (Bio-Rad). A second 2D gel run in parallel was transferred to a membrane to obtain the 2D allergenic profile (as previously described for immunoblotting).

The protein spots were distributed throughout the pH range (Figure, A). Three different spots were recognized by the patient's serum and corresponded to isoforms of the 40-kDa protein (Figure, C). These same spots were cut from the 2D SDS (Figure, A [red arrows]) and sequenced by liquid chromatography–tandem mass spectrometry, and the peptides obtained were searched for in the NCBI database for crustaceans. The search revealed 15 peptides corresponding to an MHC (accession number XP_027224354.1, www.ncbi.nlm.nih.gov/protein), with a sequence coverage of 57%.

Myosin functions alongside actin during muscle contraction. However, since it also has other functions, such as cytokinesis, cell organization, intracellular transport of organelles or other particles, and signal transduction, it is distributed throughout the body in eukaryotic cells [4].

The MHC identified in this study is a heat-stable allergen that belongs to the myosin type-2 subfamily. The structure of these proteins consists of 2 heavy chains with a molecular weight ranging from 171 to 244 kDa, which, in turn, are constituted by 3 subunits of 25, 50, and 20 kDa joined by peptide loops [5]. Each MHC is associated with 2 myosin light chains [6]. Zhang et al [4] reported highly diverse unconventional myosin genes in *Litopenaeus vannamei* and proposed that alternative splicing could produce myosin variants with different functions. This could explain why we only found the allergenic protein MHC in the heads and not in the shrimp bodies, where muscle is the main component.

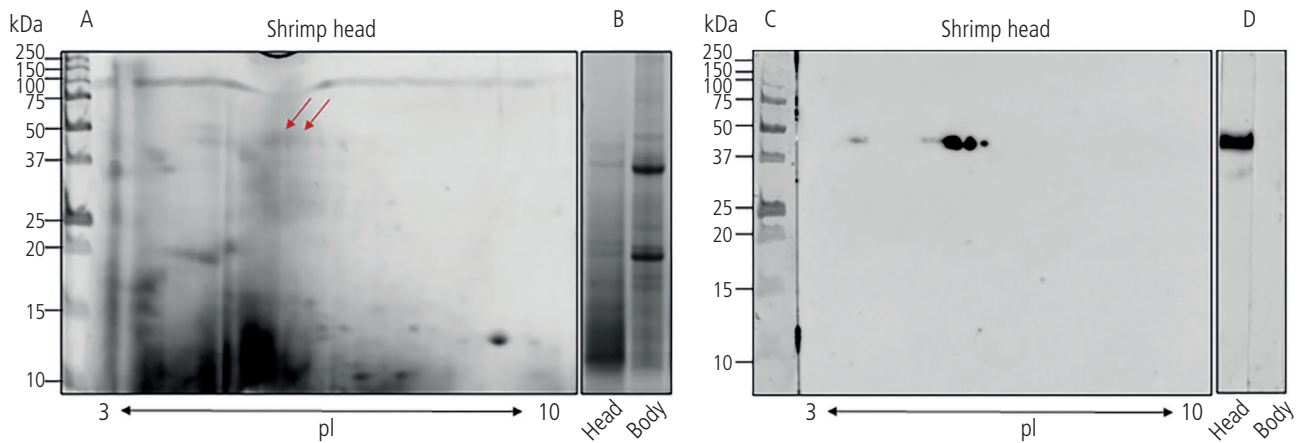


Figure. Protein and allergenic profile of the shrimp extracts. A, 2D electrophoresis of shrimp head. Spots identified by liquid chromatography–tandem mass spectrometry are pointed with arrows. B, Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of shrimp head and body. C, 2D immunoblot. D, Immunoblot of shrimp head and body. In both immunoblots, the patient serum was diluted 1/2.

Additionally, the MHC found has a molecular weight of 40 kDa, which is lower than in other MHCs.

The allergens Lit v 3 [7] and Pen m 3 are heat-resistant myosin light chain proteins [8]. In addition, MHC has been reported to be an allergen in the muscle of banana shrimp (*Fenneropenaeus merguensis*) [9]. None have been reported as exclusively head allergens.

In conclusion, we report the case of a patient with food allergy caused by the ingestion of shrimp head. The allergen involved was identified as an MHC. This is the first time a protein from this family has been reported as a shrimp head allergen. More shrimp head–allergic patients should be studied to confirm the importance of MHC as an allergen.

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Conflicts of Interest

B Saenz de San Pedro has received research support and/or speaker/consultancy fees from Takeda, Novartis, ALK-Abello, Allergy Therapeutics, Merck-Allergopharma, Meda, and GSK. She has also received funding to attend conferences/educational events from LETI Pharma, CSL Behring, Takeda, ALK-Abello, Novartis, AstraZeneca, and Roxall. She has been a clinical trial/registry investigator for LETI Pharma, Takeda, ALK-Abello, Allergy Therapeutics, Merck-Allergopharma, Roxall, and Stallergenes.

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Jerónimo Carnés
Research & Development
Laboratorios LETI, S.L.U.
Calle del Sol nº 5
28760 Tres Cantos (Madrid)
Spain
E-mail: jcarnes@leti.com