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Characterization of Major Allergens of Local Mud Crab (*Scylla serrata*)

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ABSTRACT

Crab meat is widely consumed in several countries around the world. However, when consumed, crab meats are frequent cause of allergic reactions throughout the world. *Scylla serrata* is among the most common mud crab in Malaysia. In a previous study two major allergens of mud crab at 36 and 41 kDa was identified. Thus, the aim of this study is to further identify these major allergens by a proteomic approach. Protein extract was prepared and resolved by 2-dimensional electrophoresis (2-DE). Immunoblotting was then performed using reactive sera from patients with crab allergy. Major allergenic spots were then excised from the 2-DE gel and analysed by mass spectrometry. The 2-DE profile of the extract revealed approximately >100 protein spots between pH of 4.00 to 8.00. Mass spectrometry analysis has identified the 36 and 41 kDa proteins as tropomyosin and arginine kinase, respectively. Our findings indicated that tropomyosin and arginine kinase play a major role in allergic reaction to mud crab meat among local patients with crab meat allergy, and should be included in diagnostics and therapeutic strategies of this allergy.

Keywords: *Allergy, mud crab, Scylla serrata, 2-dimensional electrophoresis, immunoblotting, mass spectrometry.*

INTRODUCTION

Crabs are common constituents in the diet of many communities worldwide [1]. However, consumption of crab meat can produce allergic symptoms in hypersensitive individuals [2,3,4]. Sensitized individuals can develop cutaneous urticaria, angioedema, asthma and even fatal or life-threatening anaphylaxis [2]. In Malaysia, a previous study indicated that about 44% of patients with asthma and allergic rhinitis suffered at least one shellfish allergy, which includes crab [4].

To date there are only few reports on identification of crab allergens at the molecular level which have been documented [2,6,7]. Leung *et al.* (1998) first reported a 34 kDa protein, designated as *Cha f* 1, as the major allergen of red crab (*Charybdis feriatus*) and identified as tropomyosin. It is well-documented that tropomyosin, a regulatory protein with a molecular mass of 35-38 kD belongs to a family of highly conserved proteins found in both muscle and non-muscle cells of all animals [8]. Tropomyosin is regarded as a pan-allergen that is implicated in IgE cross-reactivity among various invertebrates including crustaceans, mollusks, mites, helminths and cockroaches [6,7,8].

Besides tropomyosin, arginine kinase has also been reported as a new potential pan-allergen in various species of crab [9, 10, and 11]. Recently four additional new allergens of snow crab were identified as sarcoplasmic calcium-binding protein (20 kDa), troponin (23 kDa), α-actine (42 kDa) and smooth endoplasmic reticulum Ca^{2+} ATPase (113 kDa) [9]. Moreover, other potential IgE-binding proteins from crab with various molecular mass have also been reported, but have not been well-characterized [12,13,14].

Scylla serrata, commonly known as mangrove crab, mud crab or 'ketam nipah' belongs to the genus *Scylla* of family Portunidae. There is high consumer demand for *Scylla serrata*, particularly in countries comprising large Chinese communities such as Malaysia, Singapore, Taiwan and Hong Kong, where it is regarded as a delicacy, large chelae and high meat content [15]. Studies on identification of local crab allergens are very limited. Our previous studies have identified the 41 and 36 kDa protein as the major allergens of this species of crab [16]. Therefore, the aim of this study is to identify the biochemical properties of these major allergens by proteomics approach.

MATERIALS AND METHODS

Protein Extraction

Live crabs were purchased from an aquaculture centre at Port Klang, Selangor. Briefly, the crab meat was washed, minced and homogenized in phosphate buffer saline (PBS, pH 7.2), followed by overnight extraction at 4°C. The crab supernatant was centrifuged, filtered, lyophilized and stored at -20°C until use. The protein concentration of the extracts was determined using a commercial protein determination kit (Sigma, USA).

Collection of Sera

10 reactive sera from a previous study (unpublished data) were chosen to be used in this study. This research protocol was approved by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia.

2-Dimensional Electrophoresis (2-DE)

The lyophilized extract was suspended again in a rehydration buffer and applied to a 7 cm of immobilized pH 3-10 non-linear gradient strips (BioRad, USA), rehydrated overnight and focused using the IEF System (BioRad, USA). Subsequently, the strips were fractionated by molecular weight in SDS-PAGE. Protein spots map was visualized with Coomassie brilliant blue R250 staining.

Immunoblotting

The protein bands in the SDS-PAGE gel were electrophoretically transferred to nitrocellulose membrane using a Mini Transblot System (BioRad, USA). After completed, the membrane was cut, washed with tris-buffered saline (TBS) containing 0.05% Tween 20 (TTBS) and blocked for 2 hours in a blocking buffer (10% non-fat milk in TBS). The strips were then incubated overnight at 4°C with the individual patients' sera, probed with biotinylated goat-antihuman IgE (Kirkergaard and Perry Laboratories, UK), incubated in streptavidin-conjugated alkaline phosphatase (BioRad, USA) and detected using Alkaline Phosphatase Conjugate Substrate Kit (BioRad, USA). Serum from a non-allergic individual was used as a negative control.

Mass Spectrometry Analysis

The Coomassie-stained protein spots corresponding to those recognized by the above sera were manually excised, destained and submitted to a proteomics laboratory to be analyzed by mass spectrometry.

RESULTS

2-DE Profile and Identification of IgE-binding Spots

About 100 protein spots of molecular masses between 10 to 250 kDa and pI of 4.0 to 8.0 were visible with coomassie blue staining (Figure 1). The 2-DE IgE immunoblots using 10 different sera demonstrated that each subject had an individual IgE binding pattern with 12 to 40 different allergenic spots and 5 of these immunoblot results are shown in Figure 1. The most abundant IgE-binding spots of mud crab major allergens were labeled as spot number 1 (36 kDa) and 2 (41 kDa). These spots were selected for mass spectrometry analysis. No IgE-binding spots were detected by immunoblotting using a control serum from a non-allergic subject (result not shown).

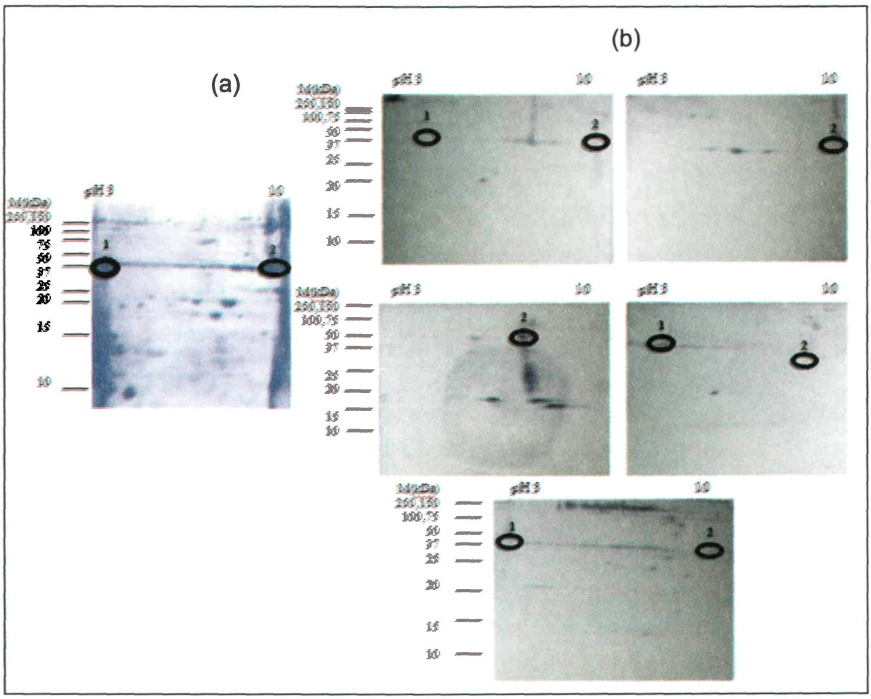

Figure 1: Two-dimensional Electrophoresis (2-DE) and Immunoblot Analysis of 2-DE Profile of Raw Extract of Mud Crab. (a) Coomassie Blue Stained of 2-DE Profile. (b) Immunoblot with Individual Patients' Sera. The Circle Represents the Protein Spot Send for Mass-Spectrometry Analysis. M, Molecular Mass Markers in KiloDalton (kDa)

Identification of Major Allergens

Two coomassie-stained protein spots (spots No. 1 and 2) were analysed after tryptic digestion by mass spectrometry. The MS/MS spectra matched significantly with known crab proteins in the databases (Table 1). Tropomyosin and arginine kinase were identified in spot No. 1 and spot No. 2, respectively.

Table 1: Combined MS and MS/MS MASCOT Search Results Summary of Mud Crab Spots (Spots 1 and 2)

Spot	MW, pI	MW, pI	Protein Identification	Organism	Accession No.	% Coverage MS/MS	Match Peptide
1	36, 4.8	32.7, 4.71	Tropomiosin	*Portunus sanguinolentus*	tr|A1YYV6	38	11
2	41, 6.9	40.2, 6.34	Arginina kinase	*Scylla olivacea*	tr|C3VUU1	32	10

DISCUSSION

Previous studies indicated that mud crab meat contains two major allergens, a heat-resistant protein of 36 kDa and a heat-sensitive protein of 41 kDa [16]. In this study, the digested peptide fragments isolated from the 36 kDa band were significantly identical to crab tropomyosin. It should be noted that several crab tropomyosin have also been reported as crab meat caused major allergens including *Cha f* 1 [7], *Eri s* 1 [2], *Chi o* 1 [17] and *Scy s* 1 [18]. This protein has also been widely identified as a major and cross-reactive pan-allergen in various species of invertebrates [8,17,19].

Contrarily, sequence homology searches of the digested peptide fragments have revealed that the 41 kDa band is significantly homologous to crab arginine kinase. Notably enzymes including arginine kinase are sensitive to high temperature, and will lead to enzyme denaturation [20]. Our finding is in accordance with recent studies which reported arginine kinase with a molecular mass of 40 kDa as a new potential allergen of snow crab (*Chionoecetes opilio*) [9] and mud crabs, *Scylla serrata* [10] and *Scylla paramamosain* [11]. At present, arginine kinase represents a new class of cross-reactive allergen in different crustacean and invertebrate species including shrimps or prawns, moth, house dust mite, cockroach and spider [17, 19, and 21]. This enzyme is a phosphagen kinase that is the key to energy metabolism in invertebrates, which catalyses the reversible transfer of the high-energy phosphoryl group from arginine phosphate to ADP to form ATP, thereby regenerating ATP during bursts of cellular activity [21].

CONCLUSION

In conclusion, this study has identified the heat-resistant major allergens of 36 kDa as crab tropomyosin and the 41 kDa heat-labile proteins corresponds to crab arginine kinase. This data could provide general insights about the properties of epitopes being responsible for eliciting allergic reaction in patients with crab meat sensitization. Hence, it is recommended that both allergens should be included for diagnostic and therapeutic approaches of crab allergy.

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