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IDENTIFICATION OF MAJOR ALLERGENIC SPOTS OF *CERITHIDEA OBTUSA* (OBTUSE HORN SHELL) BY TWO-DIMENSIONAL ELECTROPHORESIS (2-DE) AND IMMUNOBLOTTING

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Abstract: Snail can cause severe allergic reactions including anaphylactic shock. So far, only a few snail allergens at 36 and 37 kDa were reported. Thus, the aim of this study was to identify the allergenic spots of local marine snail, *Cerithidea obtusa*. The snail proteins were extracted and fractionated by two-dimensional electrophoresis (2-DE). The allergenic spots were then detected by 2-DE immunoblotting using sera from patients with snail allergy. The 2-DE fractionated the snail proteins to more than 50 distinct protein spots between molecular weights of < 10 to 250 kDa and isoelectric points (pI) of 3.0 to 10.0. Immunoblotting demonstrated that each patient had individual IgE antibodies with different binding profiles to snail allergens. At least three IgE-reactive spots were detected by each serum. Overall, 11 spots between 11 to 42kDa were able to bind to IgE antibodies. Several major allergenic spots were detected at 11, 15, 17, 25, 30 and 33 kDa. Two spots at 30 kDa with pI 5.5 and 33 kDa with pI 5.3 have been identified as highly-reactive major allergenic spots, as detected by 75 and 100% of sera, respectively. As conclusion, *C. obtusa* contains numerous major and minor allergenic spots at various molecular weights and pIs. Combination of these results with mass spectrometry and bioinformatic tools will facilitate in comprehensive identification and quantification of allergens of *C. obtusa.*

Keywords: *Cerithidea obtusa*, marine snail, allergenic spots, 2-DE, immunoblotting.

1. Introduction

Snail belongs to class Gastropoda from phylum Mollusca (Aroon *et al.,* 2005). Gastropods are univalve molluscs that are widely distributed in both freshwater and marine environments. Gastropods including snails provide as a substitute source of proteins for human besides fish. Snail is one of the mollusc groups that particularly can cause allergy after its ingestion (van Ree *et al.,* 1996). Allergy to snail can provoke respiratory symptoms (asthma and rhinitis) as well as skin reactions (urticaria and angioedema). It can also cause severe allergic reaction such as anaphylactic shock after ingestion of snail in molluscs-allergic patients (Tsapis *et al.,* 2013).

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However, there is still lack of information on snail allergens in Asia Pacific region. So far, there are several snail allergens were recognized, mainly the 36 kDa and 37 kDa that represents as minor allergens in several species of snails (Astuarias *et al*., 2002; Martin *et al*., 2005). These allergens were identified as tropomyosin, a muscle protein which plays a vital role in contractile activity and regulation of cell morphology and motility (Astuarias *et al*., 2002). Interestingly, another studies on abalone, the closest related species to snail have identified tropomyosin at 38 kDa as allergenic proteins (Lopata *et al*., 1997; Chuo *et al*., 2000). In addition, paramyosin with molecular weight of 100 kDa was also identified as allergens in abalone (Suzuki *et al.,* 2011). Paramyosin is an invertebrate-specific protein found in the core of myosin filaments and has been established as allergens in some invertebrates including house dust mites and fish parasite *Anisakis simplex* (Suzuki *et al.,* 2011).

Cerithidea obtusa (obtuse horn shell) or locally known as 'siput sedut' is an edible snail which is commonly serves as food especially in the coastal areas (Hamli *et al.,* 2013). Thus, the aim of this study was to identify the allergenic spots of *C. obtusa* by two-dimensional electrophoresis (2-DE) and immunoblotting method. This method is capable to elucidate the accumulation of multiple isoforms and post-translational modifications of the same proteins and gives relative amount of the allergens in each single spot (Abdel Rahman *et al*., 2010). In addition, the combination of 2-DE, immunoblotting, mass spectrometry and bioinformatics tools will help in comprehensive identification and quantification of allergens in *C. obtusa* (Nakamura and Teshima, 2013).

2. Materials and Method

Extraction of Snail Proteins

Live *C. obtusa* was obtained from a local seafood market. Snail proteins were extracted from the snail flesh, following the methods described by Rosmilah *et al*. (2012). Briefly, the snail flesh was homogenized in purified water, followed by an overnight extraction at 4 ºC. The homogenates were then centrifuged, filtered, dialyzed, lyophilized and stored at -20 ºC until use.

Serum Samples

Sera from four patients with snail allergy were used in this study. These sera were confirmed to have IgE antibodies specific to snail proteins in immunoblotting. Serum from a non-allergenic individual was used as a negative control. This research was approved by Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia.

Two-Dimensional Electrophoresis (2-DE)

For 2-DE gel electrophoresis, the lyophilized extract of the snail was first resuspended in rehydration buffer. 100 µg of the protein samples was then applied to 7 cm of immobilized pH 3-10 non-linear gradient strip (Biorad, USA). The first dimensional electrophoresis was performed to separate the proteins by charge with 4 steps: 250V for 15 minute, 4000V for 1 hour, 4000V for 15000 v-hr and 500V for hold. The strip containing the focused protein fractions was then separated by molecular weight using 12.5% of separating gel with a 5% stacking gel by using a Mini Protean 3 apparatus (BioRad, USA). Protein spot profile was visualized with the use of Coomassie brilliant blue R250. The pI and molecular weight of each protein spot was estimated by comparison of the protein's gel position to those 2-DE protein markers (BioRad, USA) using an imaging densitometer (BioRad, USA) and PDQuest Software (BioRad, USA).

2-DE Immunoblotting

The allergenic protein spots of the snail were identified by means of immunoblotting using sera from four snail-allergic patients. In brief, the separated protein spots were electrophoretically transferred from unstained 2-DE gel to a 0.45 mm pore size nitrocellulose using a Mini Transblot System (BioRad, USA). The nitrocellulose membrane blot was then washed, blocked and then incubated overnight with individual patient's serum. The bound IgE on the membranes was detected by incubation the strip with biotinylated goat antihuman IgE (KPL,UK) followed by incubation in streptavidin-conjugated alkaline phosphatase (BioRad, USA) and alkaline phosphatase conjugate substrate kit (BioRad, USA).

3. Result

Profile of Protein Spots

Figure 1a shows the 2-DE gel profile of *C. obtusa* proteins, stained with Coomassie blue. More than 50 distinct protein spots between <10 to 250 kDa and isoelectric point (pI) from 3.0 to 10.0 were visualized.

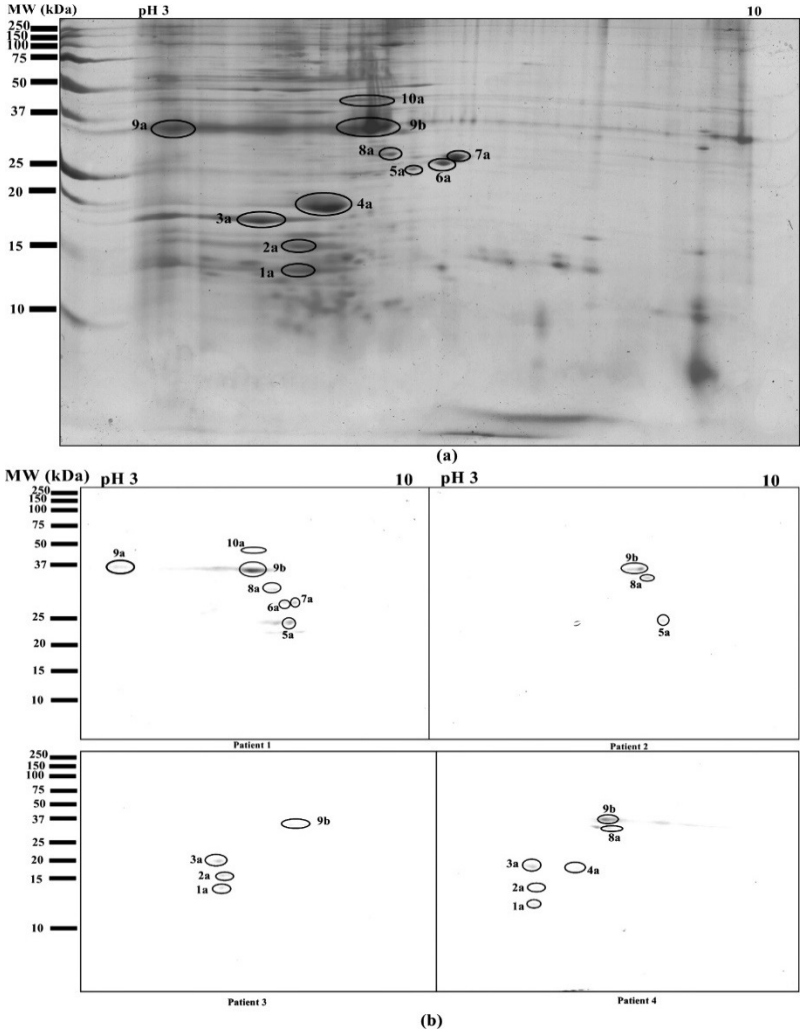

Figure 1: Two dimensional electrophoresis (2-DE) (a) and immunoblot analysis of *C. obtusa* with four individual patients' sera (patient 1 to 4). The spots in circle represent the allergenic protein spots.

Profile of Allergenic Spots

Immunoblotting of the 2-DE gels using sera from four different sera showed that each patient had individual IgE antibodies with different binding capacities (Figure 1b). At least 3 IgE-reactive spots were detected by each serum. Overall, 11 spots between 11 to 42 kDa were able to bind to IgE antibodies. Six major IgE-reactive spots were detected at 11, 15, 17, 25, 30 and 33 kDa. Two spots of 30 kDa with pI 5.5 and 33 kDa with pI 5.3 were considered as the highly-reactive major allergenic spots, as detected by 75 and 100% of the sera, respectively (Table 1). None of the proteins showed reactivity with control serum (data not shown).

Table 1: Major IgE-binding spots of 2-DE immmunoblotting of raw *C. obtusa*

No allergen	Size (kDa/pI)	Patient				Frequency (%)
		1	2	3	4	
1a	~11, 4.9			X	X	50*
2a	~15, 5.0			X	X	50*
3a	~17, 4.4			X	X	50*
4a	~20, 5.1				X	25
5a	~25, 5.9	X	X			50*
6a	~29, 6.2	X				25
7a	~29, 6.4	X				25
8a	~30, 5.5	X	X		X	75*
9a	~33, 3.3	X				25
9b	~33, 5.3	X	X	X	X	100*
10a	~42, 5.2	X				25

***** Major allergenic spots

4. Discussion

To date, allergen-specific IgE antibodies are the hallmark of the immune response to allergens and become an important diagnostic tool for allergic diseases. The used of allergenomic approach (including 2D-electrophoresis, 2D-immunoblotting and mass spectrometry) to identify and characterize the biochemical properties of allergen in *C. obtusa* are essential to understand more about the molecular nature of related allergen. The structural biology and function of allergens is important in determining the response to allergen encounter to develop sensitive and specific diagnostic and therapeutic reagents (Rolland *et al.,* 2009).

For this purpose, our study with 4 patients' sera has identified several major allergenic spots at molecular masses between 11 to 33 kDa. Two spots of 30 kDa with pI 5.5 and 33 kDa with pI 5.3 were found as highly major allergenic spots. Other studies have also reported an allergenic protein of other species of snail, *Helix aspersa* at 37 kDa (Martin *et al.,* 2005) which were identified as tropomyosin, but only as a minor allergen as it reacted to only 18% of the tested sera (Astuarias *et al.,* 2002). Therefore, we hypothesized the 30 and 33 kDa allergens of *C. obtusa* might be identical to tropomyosin, the well-known major allergens in shellfish including molluscs. Interestingly, we have also identified four spots at 11, 15, 17 and 25 kDa as major IgE-reactive spots. In addition, several minor IgE-binding spots were also detected, including the 42 kDa spot, which might corresponds to actin, a new shellfish allergens (Zailatul *et al*., 2015).

As a conclusion, *C. obtusa* contains several major and minor IgE- binding spots at various molecular weights and pIs. Mass spectrometry analysis and bioinformatics search of these

major IgE-binding spots will be performed to facilitate in comprehensive identification and quantification of allergens in *C. obtusa.*

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