

# Identification of the major allergens of *Charybdis feriatus* (red crab) and its cross-reactivity with *Portunus pelagicus* (blue crab)

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## Summary

**Background:** Tropomyosin and arginine kinase have been identified as the major allergens in multiple species of crab. *Charybdis feriatus* is an important commercial crab in this country.

**Objective:** To characterize the major allergens of *C. feriatus* using a proteomics approach and subsequently to identify the allergens involved in cross-reactivity with *Portunus pelagicus*.

**Methods:** Raw and boiled extracts of the crabs were prepared from crab meat. The protein profile of the extracts was determined by SDS-PAGE and two-dimensional electrophoresis (2-DE). Major allergens were identified by the immunoblotting test using sera from 50 patients with crab allergy. The major allergens were further identified by 2-DE immunoblotting. The major allergenic spots were then excised, digested by trypsin and identified by mass spectrometry analysis. The immunoblotting inhibition test was performed to study the cross-reactivity between red crab and blue crab allergens using sera from 20 patients with allergy to both red and blue crabs.

**Results:** At least 20 protein bands between 13 to 250 kDa were detected in the SDS-PAGE gel of raw extract, while boiled extract produced fewer protein bands. Proteins of 36 kDa and 41 kDa were recognized as the major allergens of the crab. The major allergenic spot sequences of the

36 and 41 kDa proteins were identified as crab tropomyosin and arginine kinase, respectively. All IgE-binding proteins, including both major allergens, were found to be cross-reactive with *P. pelagicus* allergens.

**Conclusions:** In addition to tropomyosin, arginine kinase was also identified as the major allergen of *C. feriatus* among our local crab-allergic patients. Cross-reactivity of this crab with *P. pelagicus* was demonstrated in this study. (*Asian Pac J Allergy Immunol* 2012;30:285-93)

**Key words:** Arginine kinase, *Charybdis feriatus*, cross-reactivity, major allergen, *Portunus pelagicus*, tropomyosin

## Introduction

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have identified shellfish, including crab, as one of the eight major sources of food allergens.<sup>1</sup> Signs and symptoms of crab allergy comprise cutaneous reactions (urticaria, angioedema, eczema), respiratory symptoms (asthma, rhinitis), gastrointestinal symptoms (diarrhoea, vomiting), and systemic reactions (anaphylactic shock).<sup>2,3</sup> Recent reports indicate an increase in crab allergy in Asian countries including Taipei,<sup>4</sup> Singapore<sup>5</sup> and China.<sup>6</sup> Our previous study also indicated a higher prevalence of shellfish allergies (including crab) than that for other food allergies among local patients with asthma and allergic rhinitis.<sup>7</sup>

Crabs belong to the order Decapoda which can be classified into two main groups, brachyuran crabs and anomuran crabs.<sup>8</sup> The majority of edible crab species belong to the infraorder Brachyura, and accordingly, a large number of brachyuran crabs are caught for human consumption in the Western Central Pacific. Among these, *Charybdis feriatus*, the red crab or crucifix crab is the commercially most important species of *Charybdis*.<sup>8</sup> This genus belongs to the family Portunidae (swimming crabs),

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Submitted date: 3/4/2012

Accepted date: 1/6/2012



which includes most of the economic crab species throughout the world.<sup>9</sup> This species has a characteristic pattern of longitudinal stripes of maroon and white on the body, usually with a distinct white cross on the median part of gastric region, and numerous scattered white spots on the legs and pincers.<sup>8</sup>

*Cha f 1* is the first and only major allergen that has been identified so far from *C. feriatius* at the molecular level, which corresponds to slow muscle tropomyosin with a molecular mass of 34 kDa.<sup>9</sup> Presently, tropomyosins from several species of crab including horsehair crab, king crab, Chinese mitten crab, snow crab and mud crab have also been reported as the major allergens at molecular level.<sup>1,2,6,10-13</sup> Most recently, arginine kinase has also been identified in mud crab<sup>14</sup> and snow crab<sup>15</sup>.

Tropomyosins are a family of closely related proteins present in muscle and non-muscle cells and, in association with the troponin complex, they play a central role in the actin-linked calcium regulatory system of muscle contraction in all invertebrates and vertebrates<sup>16</sup>, whereas arginine kinase is an enzyme that reversibly catalyzes the transfer of phosphate between ATP and various phosphagens in all invertebrates.<sup>15</sup> Because of a high degree of conservation and sequence identity, both tropomyosin and arginine kinase have been reported as strong cross-reactive pan-allergens among invertebrates including crustaceans, mollusks, mites and cockroaches.<sup>9,14-17</sup>

In addition to tropomyosin and arginine kinase, sarcoplasmic calcium-binding protein (20 kDa), troponin (23 kDa),  $\alpha$ -actine (42 kDa) and smooth endoplasmic reticulum Ca<sup>2+</sup>-ATPase (113 kDa) are four potential allergens that have also been detected in crabs.<sup>15</sup>

Although *C. feriatius* is among the most important commercial marine crab in Malaysia<sup>18</sup>, there has been no local study on characterization of allergens of this species of crab. Thus, the present study was conducted to identify the major allergens of *C. feriatius* among our local patients with crab allergy. Additionally, the *in vitro* immunoblotting inhibition test was conducted to prove the cross-reactivity between *C. feriatius* and a local Portunid crab, *Portunus pelagicus* (blue swimming crab).

## Methods

### Extraction of crab proteins

The crab proteins were extracted using our previously reported method.<sup>19</sup> Fresh crab was

purchased from a local fresh market at Port Klang, Selangor. Briefly, the crab meat was washed, minced and homogenized in phosphate buffer saline (PBS, pH 7.2) using a blender (Waring, USA). For extraction of boiled crab, the homogenates were boiled for 15 minutes. Both raw and boiled homogenates were then extracted overnight at 4°C with constant mixing (50 rpm). The crab supernatant was collected by centrifugation of the homogenates, sterile-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 from allergic subjects

Serum samples from 50 crab-allergic patients with a positive skin prick test to the raw red crab extract were used for the study. All patients were confirmed to have significantly elevated level of specific IgE to crab allergen (values above 0.35 kU/L),<sup>20</sup> using a Phadia System (Phadia, Sweden). All sera were stored at -80°C for further analysis. This research protocol has been reviewed and approved by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia.

### SDS-PAGE and immunoblot reactivity

Protein profiles and IgE-binding patterns of the raw and boiled extracts were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the immunoblotting method as described previously.<sup>19</sup> In brief, the crab proteins were treated in a denaturing Laemmli buffer and heated at 97°C for 4 minutes. Protein samples (10  $\mu$ g/ well) were run along with stained molecular weight markers (Biorad, USA) in 12.5% separating gels with 5% stacking gels using a Mini Protean 3 System (Biorad, USA). The gels were then stained with Coomassie Brilliant Blue R-250 to visualize the protein fractions, while to detect the IgE-binding proteins the immunoblotting procedure was carried out as explained previously<sup>19</sup> using sera from 50 crab-allergic patients as described above. Briefly, the unstained protein bands in the SDS-PAGE gel were electrophoretically transferred to a 0.45  $\mu$ m nitrocellulose membrane using a Mini Transblot System (BioRad, USA). After this process had been completed, the membrane was cut, washed with tris-buffered saline (TBS) containing 0.05% Tween 20 (TTBS) and blocked for 2 hours in a solution of 10% non-fat milk in TBS. The strips were then incubated overnight at 4°C with the individual patients' sera (diluted 1:5 in blocking buffer). For

detection of IgE-binding protein, the strips were probed with biotinylated goat-antihuman IgE (Kirkegaard and Perry Laboratories, UK), followed by incubation in streptavidin-conjugated alkaline phosphatase (BioRad, USA) and then in Alkaline Phosphatase Conjugate Substrate Kit (BioRad, USA). A strip without a serum sample was used as a blank, while serum from a non-allergic individual was used as a negative control in this experiment. The molecular weight of the protein fractions on the SDS-PAGE and strips were estimated by comparing the bands with the molecular weight markers using a calibrated imaging densitometer scanning and Quantity One software (Biorad, USA).

### ***2-Dimensional electrophoresis (2-DE) and immunoblot reactivity***

To detect the major IgE-binding spots of the red crab, a combination of 2-DE and immunoblotting was performed. For 2-DE gel electrophoresis, the lyophilized raw crab extract was re-suspended in a rehydration buffer. 50 µg of the protein sample was applied to a 7 cm immobilized pH 3-10 non-linear gradient strip (BioRad, USA), rehydrated overnight and focused using the IEF System (BioRad, USA). Steps to separate the proteins by charge were applied in the first dimensional electrophoresis as follow: 100 V for 1 minute, 250 V for 30 minutes, 4000 V for 2 hours and 4000 V for 10000 V-hr (V-hour). Subsequently, the strips were fractionated by molecular weight in SDS-PAGE as described above. Protein spot maps were visualized with Coomassie brilliant blue R250 staining. To identify the major IgE-reactive protein spots, 10 reactive sera were chosen for 2-DE immunoblotting, based on the results of SDS-PAGE and immunoblotting as above.

### ***Mass spectrometry and data analysis of red crab major allergen***

To identify the major allergens of red crab, mass spectrometry analysis was carried out at the Adelaide Proteomics Centre, Australia. The major IgE-binding spots were manually excised, rehydrated in 50 mM ammonium bicarbonate, diced to ~1mm<sup>3</sup> cubes, de-stained and digested with trypsin. The digested peptides were then chromatographed using an Agilent Protein ID Chip column assembly housed in an Agilent HPLC-Chip Cube Interface connected to an HCT ultra 3D-Ion-Trap mass spectrometer (Bruker Daltonik GmbH, Germany). Ionizable species were trapped and one or two the most intense ions eluting at the time were fragmented by collision-induced dissociation (CID).

The MS/MS spectra were subjected to peak detection using DataAnalysis (Bruker Daltonik GmbH, Germany), imported into BioTools (Bruker Daltonik GmbH, Germany) and submitted to Matrix Science, a search engine that uses mass spectrometry data to identify proteins from primary sequence databases.

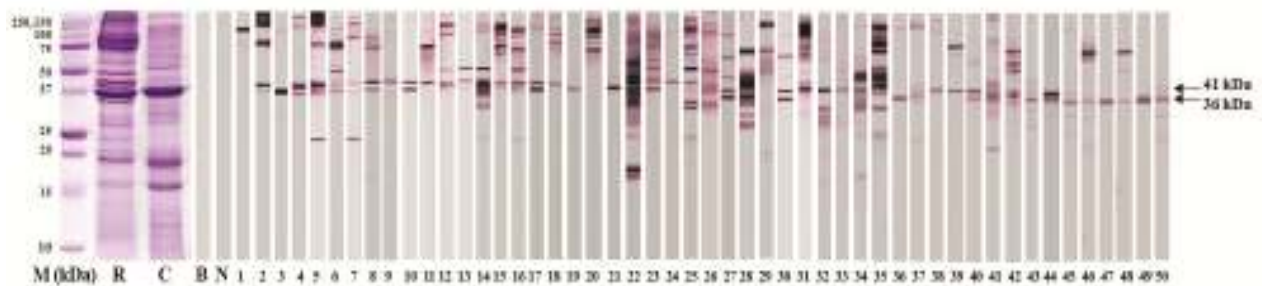
### ***Immunoblotting inhibition of red crab and blue crab***

The cross-reactivity between red crab and blue crab allergens was tested by immunoblotting inhibition experiments following the method of Leung et al.<sup>9</sup> with slight modifications. A total of 20 sera were used in this test, which were selected from 20 patients sensitized to both species of crab, as determined by the skin prick test (SPT). The lyophilized blue crab extract (stored at -20°C) was used as the inhibitor extract. Briefly, diluted sera (final dilution 1:5) were incubated with 10 µg/ml inhibitor extract (final inhibitor concentration) for 2 hours at 4°C. Strips with blotted red crab proteins were then incubated overnight at 4°C with these pre-absorbed sera. The detection of patients' IgE binding was performed by immunoblotting as explained above. Serum from a non-allergic individual was used as a negative control.

## **Results**

### ***Crab protein profiles and IgE-binding proteins***

Figure 1 and Table 1 show the SDS-PAGE gel and immunoblotting results for the red crab proteins. The raw extract exhibited at least 20 protein bands in the range of 13 to 250 kDa, while the boiled extract revealed fewer bands, as a number of protein bands between 20 to 30 kDa and 40 to 250 kDa were denatured in the boiled extract. Therefore, they were identified as heat-sensitive proteins. However, the band intensity proteins of 10 to 18 kDa were increased in the boiled extract. Immunoblotting of the raw extract demonstrated multiple IgE-binding proteins between 18 to 250 kDa, with each patient having an individual IgE-binding profile (1 to 13 IgE-binding bands). The majority of the patients' sera (82%) recognized the 36 kDa, while the 41 kDa protein was detected by 76% of patients. Therefore, these proteins were identified as the major allergen of red crab. In addition, five proteins of 50, 65, 75, 95 and 150 kDa were also recognized as potential minor allergens (frequencies of more than 25%).



**Figure 1.** SDS-PAGE analysis of the raw (R) and boiled (C) extracts of red crab and immunoblot analysis of IgE binding to raw extract using 50 patients' sera (Lane 1 to 50). Lane B and N represent a blank and a negative control, respectively. Lane M shows molecular weight markers in kiloDalton (kDa). Arrows indicate the positions of the major allergens.

### **Crab 2-DE profiles and IgE-binding spots**

The proteomics map of red crab proteins with coomassie blue staining is presented in Figure 2 (a). The 2-DE fractionated the crab proteins to at least 100 protein spots with molecular weights of between 13 and 250 kDa and pIs of 4.0 to 7.0. The IgE-immunoblotting of the 2-DE profiles demonstrated that all sera had a different IgE binding pattern with at least 10 allergenic spots as shown in Figure 2 (b). No IgE-binding spots were detected by immunoblotting using a control serum from a non-allergic subject (result not shown). The major allergens of red crab contained several protein spots and spot No. 1 (36 kDa) and No. 2 (41 kDa) were recognized by all 10 sera tested. Therefore, these spots were chosen for identification by mass spectrometry analysis.

### **Allergen identification by mass spectrometry analysis**

The MS/MS spectra of spot No. 1 and No. 2 were significantly matched with several crab proteins in the protein sequence databases (Table 2). The spot No. 1 corresponded to two types of tropomyosins, allergen tropomyosin from the three-spot swimming crab (*Portunus sanguinolentus*) and slow-tonic S2 tropomyosin from the American lobster (*Homarus americanus*). Spot No. 2 was identified as arginine kinase, identical with arginine kinase from two closely related species of infraorder Brachyura, *Pachygrapsus marmoratus* (marbled rock crab) and *Callinectes sapidus* (Atlantic blue crab).

### **Cross-reactivity of red crab and blue crab by immunoblotting inhibition**

Pre-absorption with blue crab proteins as an inhibitor of all 20 tested sera resulted in either partial or complete inhibition of their IgE reactivity

to the IgE-binding proteins (Figure 3 and Table 3). IgE reactivity to the 36 kDa major allergen of red crab was blocked completely after pre-incubation with the blue crab extract, while the IgE reactivity to the 41 kDa band was blocked either partially or completely by the inhibitor extract. In addition, the inhibition pattern was also either totally or partially inhibited IgE reactivity to other red crab minor allergens. In contrast, the control sample showed no reduction in IgE-binding intensity to all crab allergens.

### **Discussion**

The crab species *C. feriatius* is commonly consumed in our country. Although the molecular basis of the major allergen of *C. feriatius* has previously been characterized as *Cha f 1*<sup>9</sup>, to date there is no study of the identification of red crab allergens in local patients.

In SDS-PAGE analysis, a remarkable reduction in the number of resolvable protein bands of red crab was observed after boiling. Several heat-sensitive proteins between 20 to 30 kDa and 40 to 250 kDa were not detected in the boiled extract, while the band intensity of proteins between 10 to 18 kDa was increased in the boiled extract. The prominent heat-resistant band of 36 kDa which might correspond to crab tropomyosin was detected in both extracts. The loss of the protein bands in the boiled extract might be due to disruption of protein conformation by high temperature.<sup>21</sup> It has been reported that the heating process might alter proteins by inducing denaturation due to loss in tertiary and/or secondary interactions, formation of new intra- or inter-molecular bonds, aggregation, rearrangements of disulfide bonds and other conformational modifications, which can ultimately



**Table 1.** Immunoblotting analysis of raw extract of red crab using 50 sera from red crab-allergic patients.

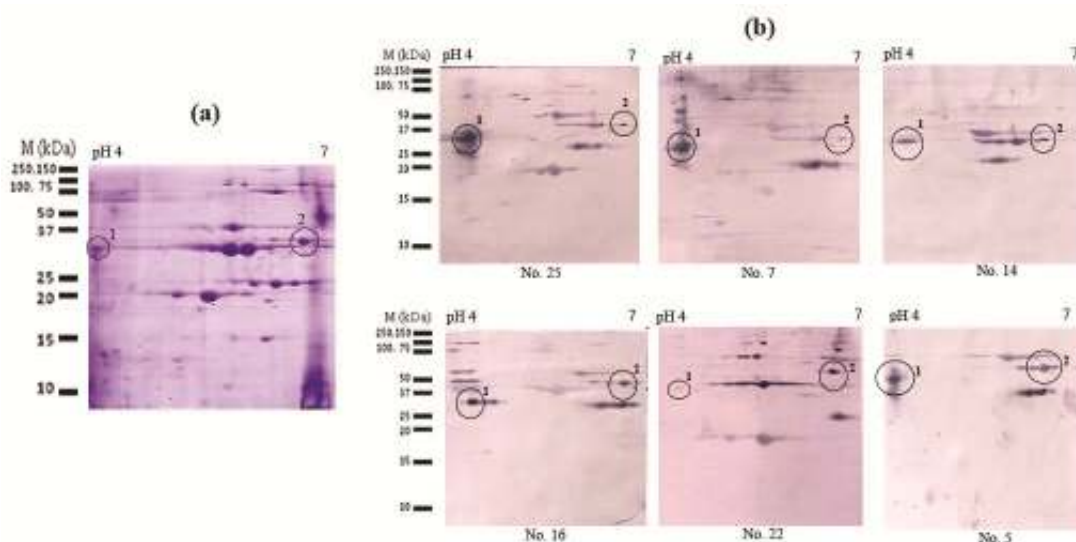
Subjects	Molecular weights (kDa)													No. of bands
	18	23	28	30	33	36	41	50	65	75	95	150	250	
1											√			1
2						√	√			√		√	√	5
3						√								1
4						√	√					√		3
5		√				√	√			√		√	√	6
6						√	√	√		√	√			5
7		√				√	√				√	√		5
8						√	√			√				3
9							√							1
10						√	√							2
11							√			√				2
12						√	√	√			√	√		5
13						√	√	√						3
14		√		√	√	√	√	√			√	√		8
15						√	√		√	√	√	√		6
16		√				√	√	√	√	√	√			7
17						√	√				√			3
18							√		√	√	√			4
19						√	√							2
20										√	√	√		3
21							√							1
22	√	√	√	√	√	√	√	√	√	√	√	√	√	13
23						√	√	√	√	√				5
24							√							1
25		√		√	√	√	√	√	√	√	√	√		10
26				√	√	√	√	√		√	√	√		8
27						√	√	√	√		√			5
28				√	√	√	√	√		√				6
29									√	√		√		3
30						√	√			√	√			4
31						√	√	√	√	√	√	√		7
32			√	√		√								3
33						√								1
34	√		√	√	√	√	√							6
35	√	√			√	√	√	√	√	√	√	√		10
36						√						√		2
37		√				√	√					√		4
38							√					√	√	3
39							√			√				2
40						√	√		√					3
41		√				√	√	√		√			√	6
42						√	√		√	√				4
43						√	√	√						3
44						√	√							2
45						√	√							2
46						√				√				2
47						√								1
48						√				√		√		3
49						√								1
50						√	√							2
N	3	9	3	7	7	41	38	15	13	22	17	18	5	
%	6	18	6	14	14	82	76	30	26	44	34	36	10	

M, molecular weight in kiloDalton (kDa); \*, Major allergen; √, IgE-binding proteins

lead to changes in allergen reactivity<sup>21</sup> by either decreasing, increasing or having no effect on allergenicity.<sup>22</sup>

The IgE binding pattern of 50 sera was heterogeneous. The number of protein bands identified by different sera varied from 1 to 13. Two protein components of 36 and 41 kDa were recognized by 41 (82%) and 38 (76%) of the 50 sera

studied, respectively. Analysis of the 36 kDa band by mass spectrometry has identified this protein as crab tropomyosin, in accordance with the reports of Leung et al.<sup>9</sup> who have successfully characterized the *Chaf 1* as the major allergen of *C. ferriatus*. *Chaf 1* was reported to have at least 90% identity with other crustaceans' tropomyosins and has 55 to 66% identity with other shellfish groups.<sup>9</sup> More recently,



**Figure 2.** Proteome profile of red crab proteins demonstrated by Coomassie blue staining (a) and immunoblot analysis of 6 sera from red crab-allergic patients (b). The spots in circles were sent for mass spectrometry analysis. M represents molecular mass markers in kiloDalton (kDa).

the tropomyosin of *Eri s* 1<sup>2</sup>, *Chi o* 1<sup>10</sup> and *Scy s* 1<sup>1</sup> have been identified as major crab allergens. This heat-stable water-soluble protein with molecular mass of 34 to 38 kDa belongs to a family of highly conserved proteins with multiple isoforms found in both muscle and non-muscle cells of all species of vertebrates and invertebrates. Allergenic tropomyosins are found in invertebrates such as crustaceans, arachnids, insects, and mollusks, whereas vertebrate tropomyosins are non-allergenic.<sup>16</sup>

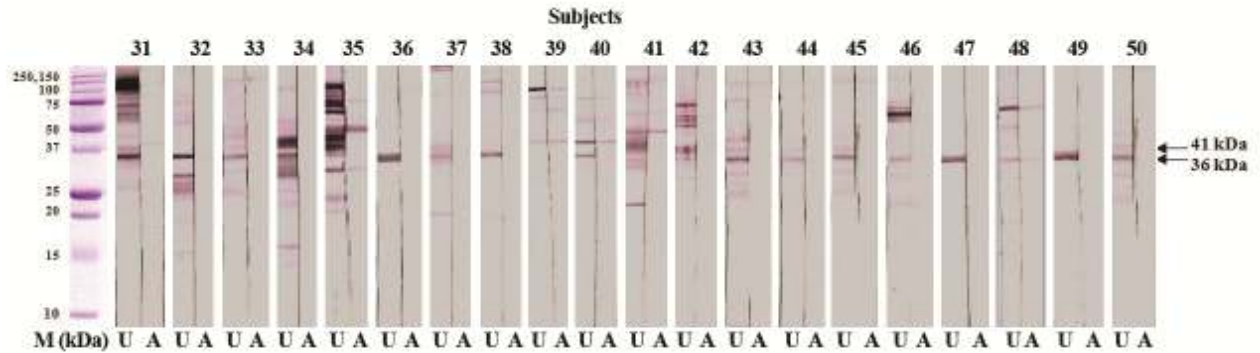
In addition to tropomyosin, analysis of the 41 kDa major allergen by mass spectrometry method revealed that this heat-sensitive band is an enzyme, identical to crab arginine kinase. It is interesting to note that enzymes generally are sensitive to heat and will denature at high temperatures.<sup>23</sup> Arginine kinase is a major invertebrate phosphagen kinase that plays an important role in regenerating ATP during bursts of cellular activity.<sup>14,15</sup> Interestingly, arginine kinase has also been recently reported as a new allergen in snow crab (*Chionoecetes opilio*)<sup>15</sup> and mud crab (*Scylla serrata*)<sup>14</sup>.

In addition to the major allergens, several high molecular weight proteins of 50, 65, 75, 95 and 150 kDa have also been recognized as potential minor allergens. On SDS-PAGE, these proteins were found to be heat-sensitive. Other studies have found nearly similar-sized allergens in crab.<sup>15,24</sup> However, the biochemical characteristics of those allergens remain unknown.

Some patients with crustacean allergy are reactive to all species of crustacean, including crabs, due to cross-reactivity and usually avoidance of all crustaceans is recommended to these patients.<sup>3,25</sup> In this study, the *in vitro* inhibition test was performed to evaluate cross-reactivity between red crab and blue crab (*P. pelagicus*), the most common marine crabs consumed in our community.<sup>18</sup> Therefore, it is not surprising to find that IgE-reactivities to the major allergens of red crab, tropomyosin protein (36 kDa) were blocked completely by the blue crab extract, while arginine kinase (41 kDa) was blocked either partially or completely by the blue crab extract, indicating highly cross-reactive major

**Table 2.** Proteins identities of the major allergens of red crab (*Charybdis feriatus*)

Spot	Observed MW, pI (kDa)	Pred. MW, pI	Protein Identification	Organism	Accession no.	% seq. MS/MS	No. of matched peptides
1	36, 4.8	32.8, 4.71	Allergen tropomyosin	<i>Portunus sanguinolentus</i>	gi 119674937	35	10
		32.9, 4.74	Slow-tonic S2 tropomyosin	<i>Homarus americanus</i>	gi 46486940	28	2
2	41, 6.9	40.6, 6.34	Arginine kinase	<i>Pachygrapsus marmoratus</i>	gi 25453078	50	23
		40.6, 6.19	Arginine kinase	<i>Callinectes sapidus</i>	gi 25453074	53	3



**Figure 3.** Immunoblotting inhibition profiles of red crab against blue crab proteins as inhibitor using 20 sera from patients identified to have allergy to both blue and red crab (patients 31 to 50). U and A were control immunoblots (using unabsorbed sera) and inhibited immunoblots (using absorbed sera), respectively. U and A were run in parallel as controls. M indicated molecular weight markers in kiloDalton (kDa). Arrows indicate the positions of the major allergens.

allergens. Notably, red crab and blue crab belongs to the same family, the Portunidae (swimming crab); thus the existence of cross-reactivity among these species might be explained by their close phylogenetic relationship. It was reported that the more closely related, taxonomically, the species are, the higher the degree of cross-reactivity.<sup>26</sup> Furthermore, it has been widely reported that cross-reactivity among invertebrates seems to be based on sequence similarities of tropomyosin IgE-binding epitopes.<sup>3,27-28</sup> World Health Organization (WHO) guidelines for the prediction of allergenicity specify that a protein can be considered to cross-react with an allergen if they share at least 35% sequence similarity in a fragment of 80 amino acids or complete identity with a peptide of 6-8 amino acids from an allergen.<sup>29</sup> Therefore, cross-reactivity between tropomyosins of species of the same class of shellfish is the norm, and the risk of reaction with a second species is 75% in patients allergic to one species of crustacean.<sup>29</sup> In addition, arginine kinase was recently proposed as a potential class new of cross-reactive pan-allergens in various invertebrates.<sup>17,22,30-31</sup>

In this study, varying patterns of cross-reactions have been observed. It is noteworthy that all sera used in our inhibition study were collected from patients sensitized to both blue and red crab according to skin prick tests (SPT). Therefore it is not surprising to observe that nearly all IgE-binding to the red crab proteins were eliminated by blue crab extracts in all patients' sera, though their clinical relevance remains unknown. This result confirms that the epitopes between blue and red crab allergens

**Table 3.** Immunoblotting inhibition analysis of red crab against blue crab proteins as inhibitor using 20 sera from patients with allergy to both red and blue crab.

Subjects	Molecular weights (kDa)												
	18	23	28	30	33	36	41	50	65	75	95	150	250
31							C	C	C	C	C	C	C
32					C	C	C						
33							C						
34		C		C	C	C	C	C					
35		C	C			C	C	P	C	C	C	C	C
36							C						C
37			C				C	C					C
38							C						C
39								P			P		
40							C	P		C			
41		C					C	C	P		C		C
42							C	C		C	C		
43							C	C	C				
44							C	C					
45							C	C					
46							C				C		
47							C						
48							C				C		P
49							C						
50							C	C					

C, complete inhibition; P, partial inhibition

are highly similar. In addition, this fact could be due to the specific inhibitory effect of other tropomyosin isoforms, tropomyosin aggregates or degradation products, or other proteins that share common reactive epitopes with tropomyosin as reported in another study.<sup>32</sup> However, partial inhibition has also been seen in this study, probably because of the existence of species-specific allergens,<sup>26,33</sup> or because of an insufficient amount of inhibitor.<sup>34</sup> It is noteworthy that the evidence suggests that serological IgE reactivity does not always result in a clinical hypersensitivity reaction<sup>35</sup>; thus rigorous clinical studies of the hypersensitivity reactions among

patients positive for tropomyosin or arginine kinase are needed to better define the frequency and severity of allergic cross-reactivity between these species of crab.

In conclusion, this paper describes the biochemical characteristics of two major allergens in the red crab *C. feriatatus*. A heat-resistant protein of 36 kDa might play an important role in allergic reaction to both raw and boiled crab, while the heat-sensitive arginine kinase at 41 kDa could be responsible for allergic reactions to the raw form in local allergy patients. Those two major allergens were found to be highly cross-reactive with the major allergens of *P. pelagicus* (blue swimming crab). Consequently, we strongly recommend that cross-reactivity phenomena between *C. feriatatus* and *P. pelagicus* should be considered in the diagnosis and therapeutic strategies of crab allergy.

### Acknowledgements

The authors would like to thank the Director General of the Ministry of Health of Malaysia (MOH) for permission to publish this paper. This study was supported by two research grants from the Ministry of Health of Malaysia (JPP-IMR 06-046 and JPP-IMR 08-010).

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