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Identification and characterisation of the major allergen of Chinese mitten crab (*Eriocheir sinensis*)

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ABSTRACT

Tropomyosin (TM) from Chinese mitten crab (*Eriocheir sinensis*) was purified to homogeneity and TM genes were amplified from three species of crab (Chinese mitten crab, mud crab and swimming crab), respectively. Sequence analysis showed that all three cloned DNA fragments had open reading frames of 855 bp, predicted to encode proteins with 284 amino acid residues. Sequence alignment revealed that the three tropomyosins share high homology to tropomyosins from other crustaceans. Chinese mitten crab TM gene was further recombined with the vector of pGEX-4T-3 and expressed in *Escherichia coli* JM109. The expressed protein revealed a band of about 62 kDa on SDS–PAGE, suggesting the successful expression of glutathione *S*-transferase–tropomyosin (GST–TM) fusion protein. Immunoblotting analysis using sera from subjects with crustacean allergy confirmed that the expressed fusion protein reacted positively with these sera, indicating tropomyosin is a major allergen of Chinese mitten crab.

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1. Introduction

IgE-mediated hypersensitive reactions to ingestion of shellfish are among the most serious forms of food allergies (Leung et al., 1998). In the United States alone, more than 6 million individuals are at risk of having hypersensitive responses upon ingestion of shellfish (Wild & Lehrer, 2005). Sensitised individuals can develop urticaria, angioedema, asthma, and even life-threatening anaphylaxis (Daul, Slattery, Reese, & Lehrer, 1994). Among shellfish, crab is assumed to be mainly responsible for severe hypersensitive reactions in China, as there are more than 600 kinds of crab in China, including Chinese mitten crab (*Eriocheir sinensis*), mud crab and swimming crab, etc. Chinese mitten crab is native to the coastal rivers near Shanghai and has long been a fashionable delicacy in China and other Asian countries. This kind of crab constitutes a substantial freshwater fishery industry in China with a production of 420,000 tons in 2004 (Zhao, Wang, Chen, Li, & Liu, 2007). Because of the increasing consumption of such kinds of crab, the occurrence of hypersensitive reactions is also increasing. The survey results on the status of food allergy in young Chinese students indicated that about 6% of subjects in the 15–24 age groups suffer at least one food allergy; the major allergenic foods are seafood, including crabs (Lu, Liu, & Yang, 2005).

The presence of a heat-stable allergen in shrimp was first identified by Hoffman, Day, and Miller (1981). Further research demonstrated that the heat-stable allergen is actually tropomyosin, a myofibrillar protein composed of two identical subunits with molecular mass of 35–38 kDa, and was regarded as the major allergen of shrimps (Daul et al., 1994; Leung et al., 1994; Shanti, Martin, Nagpal, Metcalfe, & Subba, 1993). Investigation using recombinant shrimp tropomyosin Met e 1 has demonstrated that Met e 1 can bind all the IgE reactivity of crustacean allergy sera against a 38 kDa protein both in lobster and crab by immunoblotting, further providing evidence that tropomyosin is a common allergen among crustaceans (Leung et al., 1998; Reese, Jeoung, Daul, & Lehrer, 1997). On the other hand, Hefle, Nordlee, and Taylor (1996) reported the presence of multiple IgE-reactive proteins of 25–45 kDa and 5 IgE proteins of 14 kDa or less in snow crab meat and cooking water extracts; 5 of 18 serum samples had IgE-binding to protein bands at 38–41 kDa, suggesting the involvement of snow crab tropomyosin. Thus, it is important to confirm whether the major allergen of *E. sinensis* is also tropomyosin. Furthermore, it should be noted that the cross-reactivity of *E. sinensis* allergen with those from other shellfish is not fully understood at the molecular level, since no information is available on the primary structure of the major allergen of *E. sinensis*. To further characterise the biochemical identity of crab allergens at the molecular level, we report herein the isolation, identification, and primary structure determination of crab allergen and confirmed that tropomyosin is also the major allergen of *E. sinensis*.

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2. Materials and methods

2.1. Crustacean samples and chemicals

Chinese mitten crab (*E. sinensis*) was used as a test sample; mud crab (*Scylla serrata*) and swimming crab (*Portunus trituberculatus*) were used as control. Specimens of crab were purchased alive at a local market in Xiamen. Muscle samples (raw extracts) were immediately frozen in liquid nitrogen and stored at –80 °C for molecular cloning or stored at –20 °C for tropomyosin extraction and purification.

Protein markers for SDS–PAGE and immunoblotting were from Fermentas, UAB (Vilnius, Lithuania) and New England BioLabs (Richmond, CA), respectively. Glutathione *S*-transferase (GST) affinity column was a product of Pharmacia Biotech (Uppsala, Sweden). Horseradish peroxidase (HRP)-conjugated goat anti-human IgE antibody was from Kirkegaard and Perry Laboratory (Gaithersburg, MD). Rabbit anti-shrimp (*Penaeus vannamei*) tropomyosin polyclonal antibody was prepared by our laboratory. 3, 3′-Diaminobenzidine tetrahydrochloride (DAB) was from Pierce (Rockford, IL). All other chemicals used were of the highest reagent grade from commercial sources.

2.2. Human sera

Human sera (Nos. 1–21) from 21 patients (3–39 years old) with crustacean allergy were obtained from Xiamen Women and Children Healthcare Hospital, and Hospital of Jimei University. The patients all had a history of immediate hypersensitive reaction after ingestion of crustaceans, with 76% suffering asthma, 57% allergic rhinitis, 48% atopic dermatitis, and 14% diarrhoea. These patient sera were used as a pooled positive sample or individually. Sera from five healthy volunteers (non-allergic and non-atopic individuals) without adverse reactions after ingestion of foods were pooled and used as normal negative control. All sera were stored in aliquots at −80 °C until used.

2.3. Preparation of crude cooked extracts

In general, most food allergens are proteins, with molecular masses between 10 and 70 kDa, and are stable molecules that are resistant to processing, cooking, and digestion (Lehrer, Horner, & Reese, 1996). Crude cooked extracts from Chinese mitten crab, mud crab, and swimming crab were prepared by a minor modification of a protocol as described by Shimakura, Tonomura, Hamada, Nagashima, and Shiomi, 2005. Muscle samples were homogenised with 4-fold of 0.01 M phosphate buffer (pH 7.0) containing 3% NaCl and boiled for 15 min and immediately cooled in ice-water followed by centrifugation at 18,000*g* for 20 min. The supernatant thus obtained was used as crude cooked extract. Protein concentrations of the crude cooked extracts were determined by the method of Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin (BSA) as standard.

2.4. SDS–PAGE and immunoblotting

SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was performed with 12% mini-slab gels and stained with Coomassie Brilliant Blue R-250 (CBB). Immunoblotting was carried out as described by Yu, Lin, Chiang, and Chow (2003) with minor modification. Briefly, proteins on ployacrylamide gel were electrophoretically transferred onto a nitrocellulose membrane. Non-specific protein sites were blocked with 5% skimmed milk. After washing with phosphate buffered saline (PBS; 20 mM phosphate buffer, pH 7.5, containing 0.145 M NaCl), the membrane was reacted with human serum (diluted 1:20 with 1% milk in PBS) at 4 °C for 16 h. Peroxidase-conjugated goat anti-human IgE antibody (diluted 1:2000 with 1% milk in PBS) was then allowed to react with the membrane at 37 °C for 1 h, the membrane was then washed extensively with PBST (PBS, 0.05% Tween 20). Immunodetection was carried out using DAB as substrate.

2.5. Purification of tropomyosin

Tropomyosin was purified according to Huang and Ochiai (2005) with some modifications. All purification procedures were carried out at 0–4 °C unless otherwise stated. Raw crab muscles were minced and homogenised with 10-fold of 20 mM Tris–HCl (pH 7.5) containing 50 mM KCl. After centrifugation at 8000*g* for 10 min, the supernatant was discarded and the pellet was resuspended in the above buffer, followed by homogenisation and centrifugation. The washing, homogenisation and centrifugation were repeated four more times, to remove sarcoplasmic proteins effectively. The final resulting precipitate was treated with absolute acetone three times, and dried overnight. The completely dried powder was extracted with 10-fold (v/w) of 20 mM Tris–HCl (pH 7.5), containing 1 M KCl and 10 mM 2-mercaptoethanol, overnight. The mixture was then centrifuged at 20,000*g* for 20 min and the resulting supernatant was subjected to isoelectric precipitation at pH 4.5 with 1 N HCl. The resulting pellet was dissolved in 20 mM Tris–HCl (pH 7.5) and brought to pH 7.6 with 1 N NaOH, and subjected to ammonium sulfate fractionation from 40% to 60%. The final fraction was heated in a boiling water bath for 10 min. After extensive cooling in ice water, the slight precipitate of denatured protein was spun down, leaving cooked tropomyosin in solution.

2.6. Isolation of RNA, cDNA cloning and sequencing

Total RNA was prepared from claw muscle of *E. sinensis* using TRIzol reagent (Invitrogen, Carlsbad, CA). First-stranded cDNA was synthesised with reverse transcriptase (Invitrogen) and an Oligo $(dT)_{15}$ primer, according to the manufacturer's instruction. Both sense (TS1) and antisense (TA1) primers, based on the sequences of the red crab *Charybdis feriatus* (GenBank Accession No. AF061783) and brown shrimp *Penaeus aztecus* (GenBank Accession No. DQ151457), were designed to amplify the tropomyosin of *E. sinensis*; TS1 (5′-CGC GGA TCC ATG GAC GCC ATC AAG AAG AAG ATG-3′, *Bam* HI site underlined, annealed to bases 1–24 of coding region); TA1 (5′-AGG GCG GCC GCT TAA TAG CCA GAC AGT TCG CT-3′, *Not* I site underlined, annealed to bases 855–834 of coding region).

The polymerase chain reaction (PCR) program was performed as following: 4 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 40 s at 57 °C, 40 s at 72 °C, and a final extension of 7 min at 72 °C. The PCR product was purified from agarose gel and was cloned into pGEM-T Easy vector (Promega, Madison, WI)) for nucleotide sequencing. DNA sequencings were analysed at the Invitrogen Biotechnological Co. Ltd. (Shanghai, China) with the DNA sequencer ABI Prism 3730 (AME Bioscience A/S, Toroed, Norway). At least three clones from one sample were analysed.

2.7. Expression of GST-tagged TM in Escherichia coli

The fragment of the Chinese mitten crab TM gene amplified by PCR was digested with *Bam* HI and *Not* I, and ligated into the same restriction sites of pGEX-4 T-3 (Amersham Biosciences). The recombinant plasmid was transferred into the *E. coli* strain JM109, screened and confirmed by restriction enzyme digestion, agarose gel electrophoresis, PCR and DNA sequencing. Growth and induction of transformants, purification of the recombinant protein were performed as described by the manufacturer. Briefly, positive clones were grown in Luria-Bertani medium containing 50 μg/ml ampicillin at 37 °C overnight, and inoculated (1/10, v/v) into

another fresh Luria-Bertani medium with ampicillin and induced by 1 mM isopropyl thiogalactose (IPTG) for 2 h at 37 °C. When the OD_{600} of the culture reached 0.6, the cells were harvested by centrifugation and suspended in lysis buffer and sonicated. The cell lysate was spun to pellet the debris, and the supernatant was added to GST Sepharose 4B affinity column for purification. After washing out contaminated proteins, bound proteins were eluted with reduced glutathione in 50 mM Tris–HCl, pH 8.0. Purified recombinant protein was quantified and the purity determined by SDS–PAGE. IgE reactivity of the fusion protein was also detected by reacting with human sera immunologically, as described above.

3. Results

3.1. Protein extraction and purification

Protein components of the crude cooked extract from Chinese mitten crab were analysed by SDS–PAGE and the results are shown in Fig. 1a (lane 1). Tropomyosin was further purified to homogeneity by isoelectric precipitation and ammonium sulfate fractionation. The molecular mass of the purified protein was approximately 36 kDa (Fig. 1a, lane 2). The identity and purity of the purified tropomyosin was further confirmed by immunoblotting with rabbit anti-shrimp (*Penaeus vannamei*) tropomyosin polyclonal antibody (Fig. 1b).

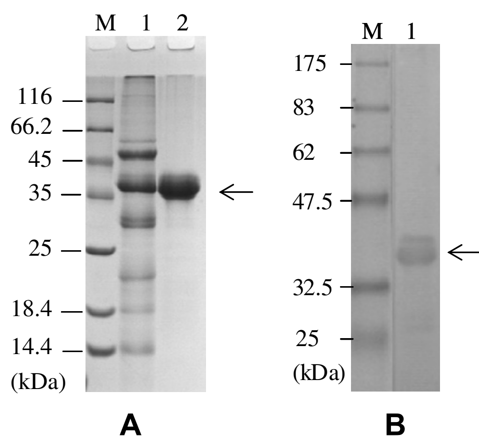

Fig. 1. SDS–PAGE and immunoblotting of tropomyosin from Chinese mitten crab muscle. (A) SDS–PAGE. M, protein marker; lane 1, crude cooked extract of Chinese mitten crab; lane 2, purified tropomyosin. (B) immunoblotting using rabbit anti-shrimp (*Penaeus vannamei*) tropomyosin polyclonal antibody. M, prestained protein marker; lane 1, purified tropomyosin.

3.2. Reactivity of IgE in patients' sera with crude cooked extract of crab muscle

To identify the IgE-binding proteins in crab muscle, crude cooked extract of crab muscle was used as antigen and investigated by immunoblotting. Sera samples from 21 crustacean-allergic patients all reacted with the crude cooked extract (Fig. 2, lane 1–21). Though more than 7 protein bands with molecular masses ranging from 25 kDa to 190 kDa could be identified as reacting with the crustacean-allergic patients' sera, the predominant band was the protein with size of around 36 kDa that was recognised by all 21 patients. The remaining IgE-binding components with various molecular masses, such as 27, 45, 55, 62 and 105 kDa, however, were detected at lower frequencies (Table 1). No IgE-binding protein was detected with pooled normal negative control sera (Fig. 2, lane 22). Thus, the protein with molecular mass of 36 kDa is quite possibly tropomyosin, the well-known major allergen of crustaceans.

3.3. Cloning and sequencing of crustacean tropomyosins

Three DNA fragments of tropomyosin gene of about 850 bp in length were amplified by PCR from Chinese mitten crab, mud crab, and swimming crab, respectively (Fig. 3). They were confirmed to

Table 1
The frequency of specific IgE-binding of sera from patients with Chinese mitten crab allergens identified by immunoblotting analysis

Chinese mitten crab allergen fraction	Molecular mass (KDa)	Frequency
1	27	19
2	36	100
3	45	19
4	47	14
5	55	14
6	62	9
7	73	9
8	105	29
9	130	14

The frequency of IgE-binding was calculated as follows: (Number of serum samples that demonstrated IgE-binding to each component – Number of serum samples with positive immunoblotting activity) × 100.

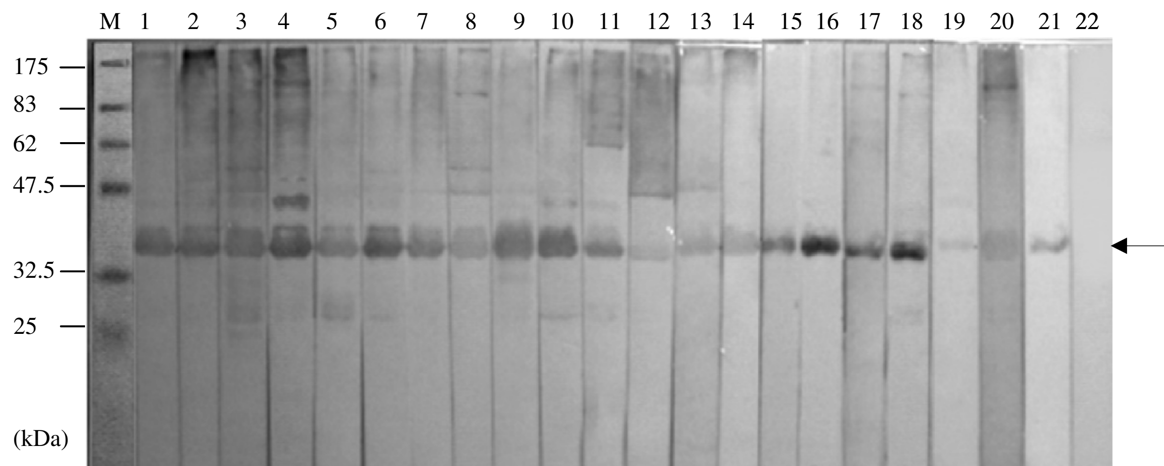

Fig. 2. Reactivity of IgE in patients' sera with crude cooked extract of Chinese mitten crab by immunoblotting. M, prestained protein marker. Lanes 1–21, sera from subjects with crustacean allergy; lane 22, pooled normal negative control sera. Arrowhead indicates the position of tropomyosin.

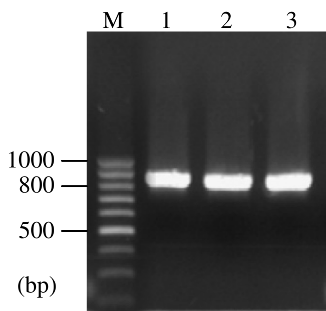

Fig. 3. PCR amplification of tropomyosin gene from three species of crab. M, DNA marker; lanes 1–3, PCR amplification products from Chinese mitten crab, mud crab and swimming crab, respectively.

be 855 bp in length by DNA sequencing and have been deposited in the GenBank database under the following accession numbers: Chinese mitten crab (*E. sinensis*), EF471314; Mud crab (*S. serrata*), EF672351; and Swimming crab (*P. trituberculatus*), EF672352.

3.4. Amino acid sequences of three crustacean tropomyosins

The deduced amino acid sequences of tropomyosins from three species of crab are shown in Fig. 4. An open reading frame of each tropomyosin encodes 284 amino acid residues. These tropomyosins are highly homologous to each another and the identity reaches more than 99.2% among the three species of crab. This high identity of sequence established the molecular basis for the immunological cross-reactivity among crab tropomyosins.

3.5. Expression and specific reactivity of Chinese mitten crab tropomyosin

The tropomyosin of Chinese mitten crab was recombined with the expression vector pGEX-4 T-3, and expressed in *E. coli* JM109 after IPTG induction. The expressed GST-tagged fusion protein exhibited a band of about 62 kDa in size on SDS–PAGE (Fig. 5) and was purified to homogeneity by GST Sepharose 4B affinity column. Because the leading protein of glutathione *S*-transferase has a molecular mass of 26 kDa, the expressed target protein tropomyo-

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Chinese mitten crab     MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKTEEDIRATQKKMQQVENELDQA   60
Mud crab                MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANLRAEKTEEDIRATQKKMQQVENELDQA   60
Swimming crab           MDAIKKKMQAMKLEKDNAMDRANTLEQQNKEANLRAEKTEEDIRATQKKMQQVENELDQA   60
Brown shrimp            MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVHNLQKRMQQLENDLDQV   60
Redspot swimming crab   MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANLRAEKTEEDIRATQKKMQQVENELDQA   60
Snow crab               MDAIKKKMQAMKLEKDNAMDKADTLEQQNKEANLRAEKTEEDIRANQKKSQLVENELDHA   60
Red crab                MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANLRAEKTEEDIRATQKKMQQVENELDQA   60

Chinese mitten crab     QEQLSAANTKLDEKEKALQNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA  120
Mud crab                QEQLSAANTKLDEKEKALQNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA  120
Swimming crab           QEQLSAANTKLDEKEKALQNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA  120
Brown shrimp            QESLLKANIQLVEKDKALSNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA  120
Redspot swimming crab   QEQLSAANTKLDEKEKALQNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA  120
Snow crab               QEQLSAATHKLVEKEKAFANAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA  120
Red crab                QEQLSAANTKLDEKEKALQNAEGEVAALNRRIQLPEEDLERSEERLNTATTKLAEASQAA  120

Chinese mitten crab     DESERMRKVLENRSLSDEERMDALENQLKEARFLAEEADRKYDEVARKLAMVEADLERAE  180
Mud crab                DESERMRKVLENRSLSDEERMDALENQLKEARFLAEEADRKYDEVARKLAMVEADLERAE  180
Swimming crab           DESERMRKVLENRSLSDEERMDALENQLKEARFLAEEADRKYDEVARKLAMVEADLERAE  180
Brown shrimp            DESERMRKVLENRSLSDEERMDALENQLKEARFLAEEADRKYDEVARKLAMVEADLERAE  180
Redspot swimming crab   DESERMRKVLENRSLSDEERMDALENQLKEARFLAEEADRKYDEVARKLAMVEADLERAE  180
Snow crab               DESERMRKVLENRSLSDEERMDALENQLKEARFLAEEADRKYDEVARKLAMVEADLERAE  180
Red crab                DESERMRKVLENRSLSDEERMDALENQLKEARFLAEEADRKYDEVARKLAMVEADLERAE  180

Chinese mitten crab     ERAESGESKIVELEEELRVVGNNLKSLEVSEEKANQREETYKEQIKTLANKLKAAEARAE  240
Mud crab                ERAESGESKIVELEEELRVVGNNLKSLEVSEEKANQREETYKEQIKTLANKLKAAEARAE  240
Swimming crab           ERAESGESKIVELEEELRVVGNNLKSLEVSEEKANQREETYKEQIKTLANKLKAAEARAE  240
Brown shrimp            ERAETGESKIVELEEELRVVGNNLKSLEVSEEKANQREEAYKEQIKTLTNKLKAAEARAE  240
Redspot swimming crab   ERAESGESKIVELEEELRVVGNNLKSLEVSEEKANQREETYKEQIKTLANKLKAAEARAE  240
Snow crab               ERAESGESKIVELEEELRVVGNNLKSLEVSEEKANQREETYKEQIKTLANKLKAAEARAE  240
Red crab                ERAESGESKIVELEEELRVVGNNLKSLEVSEEKANQREETYKEQIKTLANKLKAAEARAE  240

Chinese mitten crab     FAERSVQKLQKEVDRLEDELVNEKEKYKSITDELDQTFSELSGY                  284
Mud crab                FAERSVQKLQKEVDRLEDELVNEKEKYKSITDELDQTFSELSGY                  284
Swimming crab           FAERSVQKLQKEVDRLEDELVNEKEKYKSITDELDQTFSELSGY                  284
Brown shrimp            FAERSVQKLQKEVDRLEDELVNEKEKYKSITDELDQTFSELSGY                  284
Redspot swimming crab   FAERSVQKLQKEVDRLEDELVNEKEKYKSITDELDQTFSELSGY                  284
Snow crab               FAERSVQKLQKEVDRLEDELVNEKEKYKNIADEMDQAFSELSGF                  284
Red crab                FAERSVQKLQKEVDRLEDELVNEK....................                  264
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Fig. 4. Amino acid sequence alignment of crustacean tropomyosins. The crustacean names and their corresponding GenBank accession numbers are as following: Chinese mitten crab (*Eriocheir sinensis*), EF471314; mud crab (*Scylla serrata*), EF672351; Japanese blue crab (*Portunus trituberculatus*), EF672352; brown shrimp (*Penaeus aztecus*), DQ151457; Chinese spiny lobster (*Panulirus stimpsoni*), AF030063; sand shrimp (*Metapenaeus ensis*), U08008; redspot swimming crab (*Portunus sanguinolentus*), EF143836; snow crab (*Chionoecetes opilio*), AB270634; red crab (*Charybdis feriatus*), AF061783; The IgE-binding epitopes as proposed from brown shrimp tropomyosin are boxed.

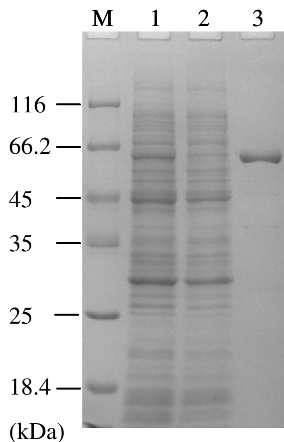

Fig. 5. SDS–PAGE of the expression of GST–TM fusion protein. M, protein marker; lane 1, cell lysate of TM-pGEX-4T-3 after IPTG induction; lane 2, cell lysate of TM-pGEX-4T-3 without IPTG induction; lane 3, purified GST–TM fusion protein.

sin was estimated to be 36 kDa by SDS–PAGE, which is in accordance to its native form (Fig. 1, lane 2).

To identify the IgE reactivity of the recombinant fusion protein (GST–TM), the *E. coli* cell lysate was used as antigen, followed by immunoblotting. Sera samples from 9 crustacean-allergic patients were incubated with the cell lysate. As shown in Fig. 6, all 9 positive sera from subjects with crustacean allergy positively reacted with the protein of 62 kDa, which is the actual position of GST–TM (Fig. 6, lane 1–9). Though other IgE-binding components with various molecular masses, such as 27, 32, 33, 83, and 85 kDa were also displayed, no positive reaction was detected by GST blank control (glutathione *S*-transferase, about 26 kDa) with pooled positive sera of crustacean allergy (Fig. 6, lane 10), suggesting reactivity to the 62 kDa protein band is mainly caused by tropomyosin. Meanwhile, no IgE-binding protein was detected by GST–TM fusion protein with pooled normal negative sera (Fig. 6, lane 11), indicating the expressed 62 kDa fusion protein is an ideal antigen for tropomyosin allergy detection.

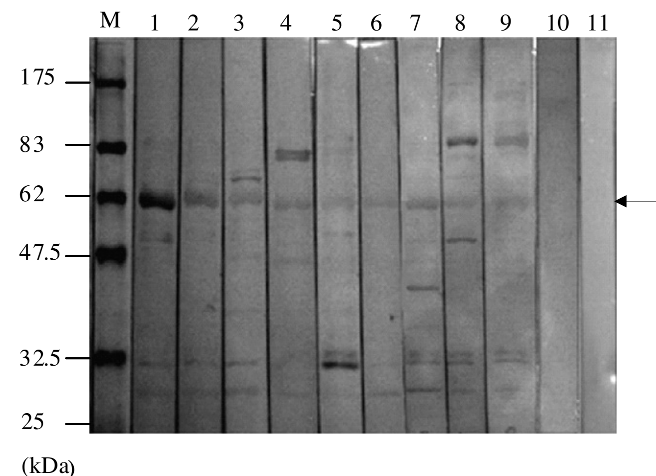

Fig. 6. IgE reactivity of sera from subjects with crustacean allergy against cell lysate of TM-pGEX-4T-3 by immunoblotting. M, prestained protein marker; lanes 1–9, sera from subjects with crustacean allergy reacted with GST–TM fusion protein; lane 10, pooled positive sera of crustacean allergy reacted with GST as control; lane 11, pooled normal negative control sera reacted with GST–TM fusion protein. Arrowhead indicates the position of GST–TM fusion protein.

4. Discussion

Consumption of seafood can produce allergic symptoms in susceptible individuals and crustacean allergies are one of the most frequently reported causes of allergic reactions. However, to date, only few reports on the identification of crab allergens and comparative analysis with other seafood allergens have been documented (Lehrer, Ayuso, & Reese, 2003). Leung et al. (1998) first reported a 34 kDa protein, designated as Cha f 1, as the major allergen of crab and identified it as tropomyosin based on nucleotide and amino acid sequence comparison.

Some commercially-available crab allergen preparations are prepared from multiple species or genera, and their reproducibility and reliability may be not adequately evaluated (Crevel, 2002; Poms, Klein, & Anklam, 2004). Thus, it is important to obtain pure allergens for improving current diagnostic and therapeutic agents. In this study, native tropomyosin from Chinese mitten crab was purified to homogeneity. Tropomyosin genes from three kinds of crab (Chinese mitten crab, mud crab, and swimming crab) were amplified and sequenced. Interestingly, though Chinese mitten crabs live in fresh water while mud crabs and swimming crabs live in salt water, all three tropomyosin genes are of 855 bp in size encoding 284 amino acid residues. Nucleotide sequence analysis revealed that Chinese mitten crab tropomyosin shares extensive similarity in amino acid composition and peptide sequence identity with that from other species of crab (Fig. 3). It is identical with that of *P. sanguinolentus* (100%) and is also significantly homologous to the *Homerianus* fast muscle tropomyosin Hom a 1 at 92.4% and *Panulirus stimpsoni* tropomyosin Pan s 1 at 91.4% (Fig. 4).

The tropomyosin of Chinese mitten crab was recombined with the vector pGEX-4T-3, and expressed in *E. coli* JM109. The fusion protein was soluble and exhibited about 62 kDa in size on SDS–PAGE, identical to the predicted molecular mass of GST–TM (Fig. 5). The expressed GST–TM was recognized by IgE sera from crustacean-allergic individuals by immunoblotting, indicating that the recombinant protein has allergenicity in expressing its IgE determinants (Fig. 6). The data of immunological reaction and sequence analysis clearly show that similar to other crustaceans, the 36-kDa tropomyosin is the major allergen of Chinese mitten crab. Compared with native crab tropomyosins which should be soluble in high concentration of salt and may affect their binding capacity to IgE antibodies, the major part of the GST–tagged recombinant protein (GST–TM) is water-soluble, and the expression output of the recombinant protein in *E. coli* JM109 is higher (20 % of all bacterium proteins).

Seafood allergen is a major cause of allergic reactions in adults (Hamada, Nagashima, & Shiomi, 2001; Motoyama, Hamada, Nagashima, & Shiomi, 2007b). The identification and characterisation of the major allergen in crabs will facilitate not only the elucidation of cross-reactions to crustaceans but also the advance of the diagnosis and treatment of seafood allergy (Hamada et al., 2003; Motoyama, Suma, Ishizaki, Nagashima, & Shiomi, 2007a; Motoyama, Hamada, Nagashima, & Shiomi, 2007b). The 36 kDa tropomyosin allergen in Chinese mitten crab as identified in the present study will benefit further allergic studies not only in this species of crab but also in other species of crab from fresh water. Also, the existence of other potential allergens in Chinese mitten crab, such as the 40 kDa arginine kinase reported by Yu et al. (2003), is noteworthy.

Acknowledgements

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