

Sequence analysis and expression of a cDNA clone encoding tropomyosin in *Sinonovacula constricta*

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Abstract Shellfish can cause severe anaphylactic reactions. Tropomyosin has been assumed partly responsible for the cross-reactivity among shellfish and other invertebrates. In this study, cDNA of *Sinonovacula constricta* was amplified by RT-PCR and 3'-RACE from total RNA. The obtained tropomyosin cDNA included an open reading frame coding for 284 amino acids. The deduced amino acid sequence of the corresponding protein shared high identity with other allergenic tropomyosins. Expression of the recombinant tropomyosin was carried out in *Escherichia coli* BL21(DE3) using vector PET28a and the purification of the recombinant protein was performed via affinity chromatography. IgE reactivity of recombinant tropomyosin was investigated by immunoblot and the sensitized percentage was 36% which indicated that tropomyosin was the minor allergens in *S. constricta*. Moreover, the character of the purified protein was analyzed by MALDI-TOF-MS.

Keywords MALDI-TOF-MS · Mollusc · Shellfish · *Sinonovacula constricta* · Tropomyosin

Introduction

A food allergy is an immunological response to a food protein. It is estimated that about 1–2% of adults and about

4–8% of children are affected by food allergies and that the prevalence is still rising [1–3]. Food allergy can be triggered by tiny amounts of protein allergens in food and can sometimes cause life-threatening reactions [4].

Two invertebrate groups, Crustacea and Mollusca, are generally termed as 'shellfish' and are common constituents in the diet of many populations. Shellfish is one of the most common food allergens and is among the eight foods that account for over 90% of food allergies [5]. In the US alone, more than 250,000 individuals are at risk of developing hypersensitive responses upon ingestion of shellfish [6]. In Singapore, a study discovered that the most common food allergens in patients, including 49 Chinese (66.2%), five Malays (6.8%), eight Indians (10.8%), and 12 (16.2%) other racial denominations, were seafood crustaceans, molluscs and bird's nest [7].

Tropomyosin, a kind of muscle protein, is highly conserved in invertebrates and vertebrates. The invertebrate tropomyosins are highly allergenic and are responsible for a significant proportion of invertebrate allergies [8].

The major shrimp allergen was first identified as tropomyosin by Shanti et al. [9]. Subsequent studies by two other groups confirmed that tropomyosin is the major heat-stable shrimp allergen [10, 11]. Since then, tropomyosins have been confirmed as major allergens in many other crustaceans, insects and arachnids, such as crab, lobster, molluscs, mussel, squid, cockroach, and house dust mites [12–20]. Furthermore, tropomyosins have been termed as pan-allergens in invertebrates due to their conserved structure [21, 22].

The recent reports suggested that tropomyosin is an allergen in mollusca. A significant portion of populations allergic to shellfish are also hypersensitive to mollusks [23, 24]. However, the available information at a molecular level, such as DNA sequence, gene expression, and IgE-binding to mollusc tropomyosin, is very limited.

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Sinonovacula constricta, a kind of mollusca, is widely found in intertidal zones and estuarine water in China, Japan, and Korea. It is an important commercial bivalve and a popular mollusca food in China. In the present study, cDNA and deduced amino acid sequence of tropomyosin were obtained from *S. constricta*. The recombinant protein of tropomyosin was expressed in *E. coli* and purified by Ni⁺ affinity chromatography. The IgE-binding capabilities of recombinant protein were studied by immunoblotting experiments. Moreover, the purified protein was identified by MALDI-TOF-MS.

Materials and methods

Patients' sera

Sera samples used in this study were collected from sea-food-allergic patients with a positive case history of immediate-type allergic reaction to *S. constricta*. Only the sera from patients with a positive skin prick testing result to *S. constricta* (>7 mm²) was used in the study. Sera from nonallergic subjects served as negative control. All sera were kindly supplied by Shenzhen People's Hospital and all samples were stored at −80°C.

Molecular cloning and sequence analysis

Total RNA was prepared from *S. constricta* with RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. The reverse transcription was carried out using AMV First-strand cDNA synthesis Kit (BBi). The cDNA obtained was amplified by polymerase chain reaction (PCR) using a pair of degenerate primers designed on the basis of known allergenic tropomyosins (p5: 5'-ATGGATGC(C/A)ATCAAGAAG AAG-3', p3: 5'-T(T/C)AGCC AGT CAG TTC GC-3'). The amplification was performed with *Ex-Taq* DNA polymerase (Takara) as described by Song et al [25]. The PCR amplified DNA fragment was gel-purified and subcloned into pMD18-T vector (Takara). The constructed plasmids were transformed into *E. coli* Top10. The positive clones were sequenced by Sangon Biotechnology company (Shanghai, China) using the ABI 337 sequencer.

Rapid amplification of 3'-cDNA end was performed with the 3'-full RACE Core set (TaKaRa) according to the method described by Song et al [25]. The amplified products were cloned into the pMD18-T vector and transformed into *E. coli* Top10, the positive clones were sequenced. The full-length cDNAs were obtained by the overlapping of the truncated gene and its 3'-end.

Analysis of the derived nucleotide and its deduced amino acid sequences was performed using the BLAST network service of the NCBI (National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov>). A homology search in GenBank was performed using the NCBI Blast program and the alignment of amino acid sequences was generated by CLUSTAL W (1.83) multiple sequence alignment program.

Protein expression, purification, and immunoblotting

Tropomyosin-encoding cDNA was subcloned into the *Nde* I and *EcoR* I restriction sites of the expression vector PET28a and expressed in *E. coli* BL21(DE3). The cells were grown in LB-medium supplemented with 50 µg/ml kanamycin to an absorbance of 0.6 at a wavelength of 600 nm. Expression of *S. constricta* tropomyosin was induced by adding isopropyl-D-thiogalactopyranoside (IPTG) to LB-medium at a final concentration of 1 mM and the incubation was continued for 4 h at 37°C. Cells were harvested by centrifugation and resuspended in 20 mM Tris-HCl, 100 mM NaCl, pH 7.5. After three cycles of freeze-thawing, cells were sonicated to obtain the lysate, insoluble material was removed by centrifugation. Recombinant protein was purified by affinity chromatography using the Ni²⁺ as solid phase. The products were dialyzed three times against 0.02 M Tris-HCl buffer and then freeze-dried.

Protein from *E. coli* lysate and purified recombinant tropomyosin were analyzed by SDS-PAGE. For immunoblot analysis, recombinant tropomyosin (0.5 µg/cm slot) and protein extracts of *S. constricta* were electrically transferred onto the nitrocellulose membranes. The membranes were blocked overnight with 2% bovine serum albumin solution (BSA) at 4°C, then reacted with 1:5-diluted allergic patients' sera for 2 h at 37°C. The biotin-labeled goat anti-human IgE antibody (KPL) were used as the secondary antibody, followed by incubation with the streptavidin-conjugated-HRP at 37°C for 2 h. The blotting bands were finally visualized with diaminobenzidine (DAB) chromogenic substrate solution.

Protein identification by MALDI-TOF

Sample preparation for MALDI-TOF-MS analysis was performed according to the method of proteomic protocols for mass spectrometry (2005 edition) supplied by Bruker Daltonics which were adapted from the methods of Shevchenko et al [26]. In brief, protein bands of interest were excised and destained. After dried by speed vacuum, gel pieces were treated with 10 mM dithiothreitol in 25 mM

NH_4HCO_3 for 45 min at 56°C and with 55 mM iodoacetamide in 25 mM NH_4HCO_3 for 30 min at room temperature in the dark. Then the gel was treated with 25 mM NH_4HCO_3 in 50% acetonitrile for 15 min. This procedure was repeated, followed by enzymatic digestion with trypsin at 37°C overnight. Digested peptides were eluted with 100% acetonitrile which contained 0.1% TFA. Eluates were analysed by matrix-assisted laser desorption ionization-time-of flight mass spectrometry (MALDI-TOF). The peptide mass values obtained by MALDI-TOF were used as inputs to search corresponding proteins with the Mascot search engine (Matrix Science, London) (<http://www.matrixscience.com>).

Results

Cloning and sequence analysis of the *S. constricta* tropomyosin

By amplification of cDNA from first strand synthesis products with degenerated oligonucleotide primers, a clone approximately 850 bp were obtained from *S. constricta*. The uncertain 3'-end of cDNAs were determined by using 3'-RACE methods with an overlap of 329 bp. The cDNA sequence was submitted to the NCBI GenBank under the accession number **EU082209**.

The entire cDNA length was about 1,170 bp containing a complete 855 bp open reading frame, a 3'-untranslated region and a poly (A) tail, respectively (Fig. 1). The length of the deduced amino acid sequences of tropomyosin was 284 amino acid residues and its predicated molecular mass was 32.96 kDa and its isoelectric point was 4.66.

Comparison of the deduced amino acid sequence of tropomyosin revealed a high degree sequence identity with previously described allergenic tropomyosin from shellfish, dust mite and cockroach et al. (Fig. 2).

Expression and purification of recombinant *S. constricta* tropomyosin protein

For expression of the recombinant tropomyosin, the complete coding cDNA sequence including the start and the stop codons was cloned into the PET28a expression vector and then transformed into *E. coli* BL21(DE3). After inducing with IPTG for 4 h at 37°C , the recombinant tropomyosin protein was harvested. The soluble material which includes most of the target protein was chromatographed over a Ni^{2+} Sepharose affinity column. After elution with 200 mM imidazole, recombinant tropomyosin was isolated with high degree of purification (Fig. 3). The yield of the purified protein was 5.495 mg/L as measured by the Bradford assay.

Sensitization to *S. constricta* tropomyosin

Serum samples taken from 11 patients which have adverse symptoms after ingestion of *S. constricta* were investigated for IgE reactivity to recombinant tropomyosin and protein extracts of *S. constricta* by immunoblot experiments. A pooled serum of ten people with no allergic reaction was used as control. In the immunoblot experiments, IgE antibodies from 4 out of 11 serum samples (36%) reacted with the purified recombinant tropomyosin (Fig. 4), and all of

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ATGGATGCCATCAAGAAGAGATGCAGGOCATGAAGATAGAGAAGGAGAAATGCTCTAGACAAGTCAGAGCAGTTGGAAAAATAAATTAAAGCAAAATTGAGG 100
M D A I K K K M Q A M K I E K E N A L D K S E Q L E N K L K E I E
ATGTTAAGGTCAAGATAGAGGAGGACCTTAOCCTCTCTCCAGAAGAAATACACCAACCAGGAGAATGAGTACGACAAAGTCAACGAGCAGTTCAACGAGAG 200
D V K V K I E E D L T S L Q K K Y T N Q E N E Y D K V N E Q F N E S
CACTGTCAAGCTGAGGCCTCTGAGAAGAGAGTCACTGAGTGTGAAGATGAGATCAAGGGCTTCACTCCGAGAATCCAGCTGTTGGAAGATGAATTGGAG 300
T V K L E A S E K R V T E C E D E I K G F T R R I Q L L E D E L E
CGCACAGCAGCAAGGOCGAGGAAGCTGTCTTAAGTTGCAAGAGGOCCTCAAGGCTGCAGATGAGAGCGAGAGAGGAAGAAAGGTGCTGAGAGCAGAA 400
R T Q Q K A E E A V L K L E E A S K A A D E S E R G R K V L E S R
GCATTGCGATGACGACAGAATGACAAGTTGGAAGGATGTGAAGGATTCCAAATAOCTTGCTGAAGAGGCTGAOCGCAATACGATGAGGCGGCGCG 500
S I A D D D R I D K L E K D V K D S K Y L A E E A D R K Y D E A A R
TAAGCTTGCTATCACTGAAGTCGATCTTGAACGTGCGAGACACGCTTGAAGCGGCTGAATCCAAAATTACTGAACTCAGCGAGGAGCTGCAGGTGCTT 600
K L A I T E V D L E R A E T R L E A A E S K I T E L S E E L Q V V
GGTAATAACTGTAAAGCTCTTTCAGAAAGCGCTTGACCAAGCTTTCACAGAGAGAGGACAGCTAOCAGGAGACTATCCGCGACTTGACACAGAGACTCAAGG 700
G N N C K A L Q N A V D Q A S Q R E D S Y E E T I R D L T Q R L K
ATGCTGACAAACGCTGCTGCTGAAGCTGAACGTGTGTCAACAAGCTGCAGAAGGAAGTTGACCGTCTGGAAGATGAAGTCTTCAAGAGAAGGAGAAATA 800
D A E N R A A E A E R V N K L Q K E V D R L E D E L L Q E K E K Y
CAAGCAATCAGTGATGAACCTTGATCAAAATTTGCTGAATTGGCTGGTATGTGAGGAGACAACCTACAACCACACCACATCAACATCGACGTCAAG 900
K Q I S D E L D Q T F A E L A G M *
atatcaacgctgcgtcttttatgccccttttcgctaatactttgacactatgccatttagagtggaaactttcggaatatttcagatgtttgcatagat 1000
gttgtaactccatttagttaagaaaagggtgtttgtgttaaagctatatattatgtgtaaagctgaaagatgtccaagaaaaactgtgactgctttgtggc 1100
ttgtatttgtgacacgaacttgtaatacttggtgaaaaaagacattcaattaaactaacacaaaaaataaaaaaataaaaaaataaaaaaataaaaaa 1200

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Fig. 1 Nucleotide sequence (upper) and deduced amino acid sequence (lower) of *S. constricta* tropomyosin. Nucleotide numbers on the right

ABU53681.1	MDAIKKKQAMKIEKENALDKSEQLNKLKEIEDVVKVKEEDLTSLQKKY	50
Q9GZ69	*****VDR***Q*LA**M*Q***DT*TA**A*L**E*FNE***L	50
P91958	*****V***M*****RA***Q**R*TA**A***D*Y*****S	50
BAE54430.1	*****L***M***V*TA**T*Q**RDL**A*N*****ST***S	50
Q9GZ71	*****L***M***V*RA**T*Q**RDT**E*Q**A*****N**C	50
P43689	*****L***M***I*RA**M*Q**VRDV*ET*N*L**E*FNN***F	50
O97192	*****L***M***RA**V*Q**RDC*CN**V*****N***F	50
Q25456	*****L***M***RADT**CON**ANLRAE**T**E*IRAT***M	50
ABL89183.1	-----*L**D**M**RADT**CON**ANNRAE**S**E*VHN***RM	40
ABF51441	*****L***D***RAMC**QQA**DANLRAE**A**E*ARQ***I	50
AAL86701	*****D***M**RALLC**QQA**DANLRAE**A**E*ARQ***I	50
Q23939	*E*****L**D**I*RA**I*Q**ARDANLRAE**S**E*VRA***I	50
ABU53681.1	TNQEENEYDKVNEQFNESTVKLEASEKRVTECEDEIKGFTTRIQLEDELE	100
Q9GZ69	*AT**N***T***L*Q*ANT***N***Q*QL*SDVGC*Q*MLT***E*Y*	100
P91958	IQT**DL*NTQT*LQDVQA*Y*TT**Q*IA**H*Q**QSL**K*SM***E*D*M	100
BAE54430.1	A*L**DF*NA***L*TAANTN*****A***S**Q*H*L***E*ED**	100
Q9GZ71	A*L**DF*NA***L*Q*AMAA**T*****M*Q*VS*AT**K*TA**E*ED**	100
P43689	S*L*Q*DF*TA**GL*TAQT*****H*A*L*SDTA*L*N*****E*ED**	100
O97192	AI*L**DF*SI***L*LDANT*****KNA**I*S*TA*L*Q*****E*ED**	100
Q25456	QQV**L*QAQ*ML*SAANT*LDEK**ALQNAEG*VAALN*****E*ED**	100
ABL89183.1	QQL**DL*Q*Q*SL*LKANQ*V*EKD*AL*SNAG*VAALN*****E*ED**	90
ABF51441	QTI**L*QTQ*SL*MQVNC**EK**ALQNA*S*VAALN*****E*ED**	100
AAL86701	QQI**DL*QT*ML*MQVNA**DEKD*ALQNA*S*VAALN*****E*ED**	100
Q23939	QOI**L*Q*Q*ML*SAANT**EK**ALQTA*GDVAALN*****I*ED**	100
ABU53681.1	RTQQAEEAVLKLEASKAADSEGRKRVLESRSIADDDRIDKLEKDVKD	150
Q9GZ69	*SEE*L*NSTTE*****N***G**NSYEE***E***QL*ET	150
P91958	KSEERYTT*A*S*****N*****NLNCGN**E***Q***QL*TE	150
BAE54430.1	*SEERFSS*Q**S***D*****N***Q**E*E*E*L***QL*EE	150
Q9GZ71	*N*EERLQT*TER*****AR*****L***E***Q***QL*E	150
P43689	*SEERLQS*TE*****L***E**L**C**A*Q*E	150
O97192	*SEERLQS*TE*****L***E**L**C**A*Q*E	150
Q25456	*SEERLNT*TT**A***Q*****M*****N**LS*EE**M**A**N*Q*E	150
ABL89183.1	*SEERLNT*TT**A***Q*****M*****N**LS*EE**M**A**N*Q*E	140
ABF51441	*SEERLAT*TA**S***Q*****A*****L**E*E**M**A**N*Q*E	150
AAL86701	*SEERLAT*TA**A***Q*****A***I**K*G**E*E**M**A**N*Q*E	150
Q23939	*SEERLKI*TA*****Q*S*****M**H***T*E*E**M**C**N*Q*E	150
ABU53681.1	SKVLAEEADRKYDEAARKLAITTEVDLERAETRLAESAESKI TELSEELQVV	200
Q9GZ69	A*NV*TD**HKF*****D***V***E***T**	200
P91958	A*W***D***K*E*****A*****A*V*ID*Q*Q*TA**	200
BAE54430.1	A*W***D***K*E*****A*****A*V***E***K**	200
Q9GZ71	A*W***D***E*****A*****A*V***E***K**	200
P43689	A*W***D***E*****A*****A*V***E***K**	200
O97192	A*W***D***E*****A*****A*V***E***K**	200
Q25456	ARF*****V*****IV*A*****E*A*SC*****V***E***R**	200
ABL89183.1	ARF*****V*****IV*A*****E*A*SC*****V***E***R**	190
ABF51441	ARF*****K*****V*****IV*A*****E*A*SC*****V***E***R**	200
AAL86701	ARFM*****K*****V*****IV*A*****E*A*SC*****V***E***R**	200
Q23939	ARMM*****D*****V*****IV*A*****E*A*TC*****V***E***R**	200
ABU53681.1	GNWCKALQNAVDAQSREDSYEETIRDLTORLKDAENRAAEARVVKMLQ	250
Q9GZ69	*A*I*V***VQN*****K*S*****T***Q*V***	250
P91958	*A*I*V***VQN*****H*****T***TVS***	250
BAE54430.1	***M*S*E*ISEQE*****H***E*****T***S***	250
Q9GZ71	***M*S*E*ISEQE*****T***T***S***	250
P43689	***M*S*E*ISEQE*****T***T***S***	250
O97192	***M*S*E*ISEQE*****S***T***S***	250
Q25456	***L*S*E*VSEEEK*N***E*A*K*Q*KT*ANK**A**A**E*F***S*Q***	250
ABL89183.1	***L*S*E*VSEEEK*N***E*A*K*Q*KT*TNK**A**A**E*F***S*Q***	240
ABF51441	***L*S*E*VSEEEK*N***E*E*K*Q*KT*TNK**A**A**E*F***S*Q***	250
AAL86701	***L*S*E*VSEEEK*N***E*A*K*Q*KT*TNK**A**A**E*F***S*Q***	250
Q23939	***L*S*E*VSEEEK*N***E*A*Q*Q*KT*TNK**A**A**E*F***S*Q***	250
ABU53681.1	KEVDRLDELLQKEKQYKQISDELDTFAELAGM-	284
Q9GZ69	*****A***R***A***D*****I***Y-	284
P91958	*****A***R***A***D*****I***Y-	284
BAE54430.1	*****A***R***A***D*****I***Y-	284
Q9GZ71	*****A***R***A***D*****I***Y-	284
P43689	*****A***R***A***D*****I***Y-	284
O97192	*****A***R***A***D*****I***Y-	284
Q25456	*****V*****S*****S*****S*****Y-	283
ABL89183.1	*****V*****S*****S*****S*****Y-	274
ABF51441	*****V*****S*****S*****S*****Y-	285
AAL86701	*****V*****S*****S*****S*****Y-	284
Q23939	*****V*****S*****S*****S*****Y-	284

Fig. 2 Alignment of amino acid sequences of *S. constricta* tropomyosin with those of allergenic tropomyosin from the other molluscs, crustaceans and other invertebrates. Asterisk indicates identical amino acids, dashed lines are gaps. The sequence identities between *S. constricta* tropomyosin and other tropomyosin are included at the bottom right of figure

these four serum samples have positive reaction with the protein extracts of *S. constricta*. Furthermore the 1st sera sample which had no reaction with the recombinant tropomyosin also presented positive reaction with the protein extracts (Fig. 5). No reaction was found with sera from control subjects.

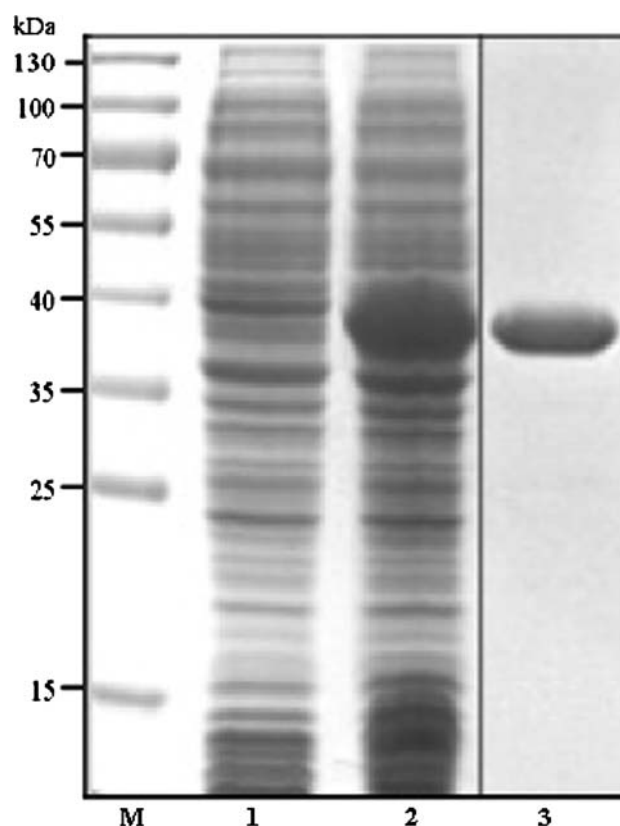


Fig. 3 Coomassie-stained sodium dodecyl sulphate-polyacrylamide gel electrophoresis of the supernatant fraction from transformed *E. coli*-lysates with PET28a vector carrying the *S. constricta* tropomyosin cDNA. M, protein molecular weight marker; lane 1, before induced; lane 2, induced 4 h; lane 3, purified recombinant tropomyosin

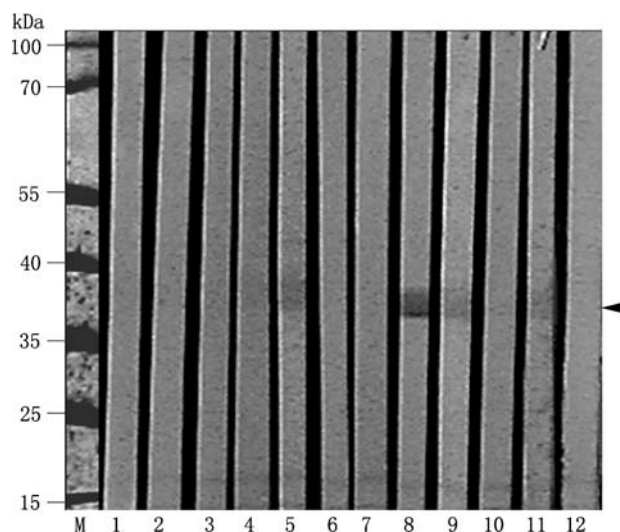


Fig. 4 IgE-immunoblot of purified recombinant *S. constricta* tropomyosin with individual sera (1-11) from patients with allergy to *S. constricta* and with the control serum (12). The 5th, 8th, 9th and 11th serum samples have positive reaction with recombinant *S. constricta* tropomyosin

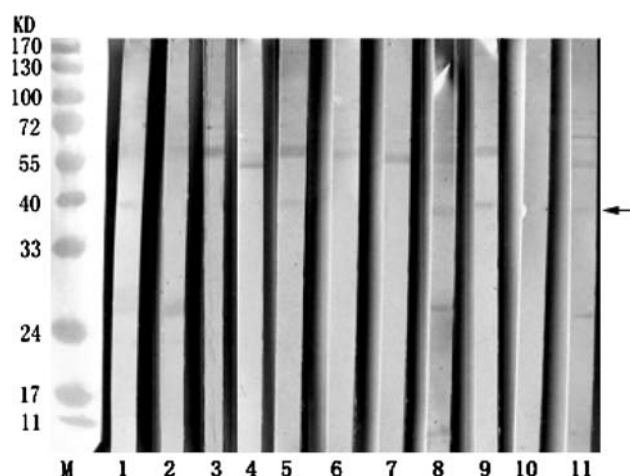
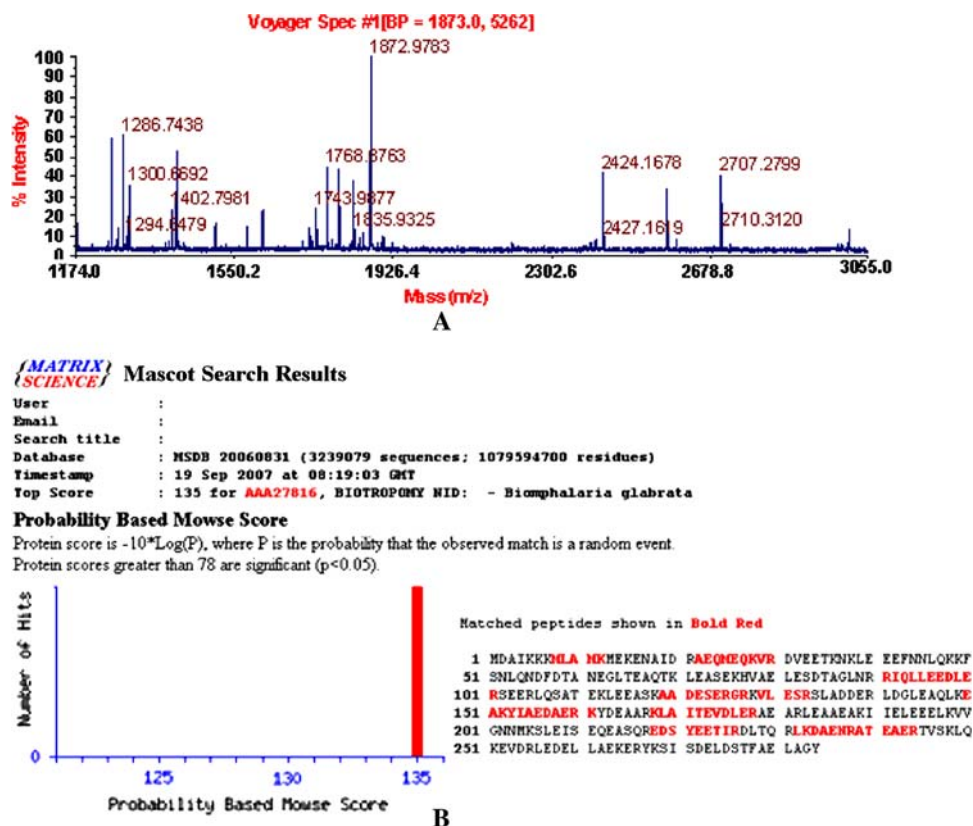


Fig. 5 IgE-immunoblot of *S. constricta* protein extracts with individual sera (1–11) from patients with allergy to *S. constricta*. The 1st, 5th, 8th, 9th and 11th serum samples have positive reaction with natural tropomyosin from *S. constricta* extracts

Identification of the recombinant protein by mass spectrometry analysis

The protein band of interest was excised, cleaved with trypsin and analysed by MALDI-TOF-MS. The peptide masses were submitted to the SwissProt protein database using MASCOT software and searched against all known proteins. Molluscs tropomyosins were the top matches,

Fig. 6 Typical MALDI-TOF spectrum (a) and Mascot search results (b) of recombinant *S. constricta* tropomyosin



with tropomyosin from the *Biomphalaria glabrata* (GenBank accession no. **AAA27816**) being the top hit (Fig. 6), followed by *Helix aspersa* (GenBank accession no. **CAB38044**) and *Haliotis asinina* (GenBank accession no. **AAP85232**). The validity of the results for protein identification was measured by the probability based MOWSE score of Mascot. In this study the Mascot score, which greater than 78 is significant, was 135, 113, and 100 respectively.

Discussion

Hypersensitive reaction to seafood is one of the most common food allergies. In recent years, the growing production and consumption of seafood and the concomitant increasing risk of seafood allergy in the population make it important to identify the mechanism of seafood allergens at the molecular level.

Shellfish including crustaceans and mollusca is a subset of seafood. Most types of shellfish can cause hypersensitive reaction. Furthermore, a person allergic to one kind of shellfish is usually advised to avoid other types of shellfish, for one sensitive to one type of shellfish is most likely to react to other shellfish [27–29]. The reason that individuals allergic to some type of crustacea are at risk for reacting to other type shellfish maybe is that there is a

relatively high risk of cross-reactivity among different types of shellfish.

Tropomyosin has been suggested to be the cross-reacting allergen between shellfish, insects, and mites. Ree et al. has reported that induction of IgE during mite immunotherapy might occasionally cause allergy to foods of invertebrate animal origin such as shrimp [30]. The studies of Fernandes et al. also indicated that IgE antibody reactivity to a shellfish allergen can occur in an unexposed population of individuals due to the cross-reacting tropomyosins [31]. So it is necessary to investigate the shellfish allergen tropomyosin.

During the past decade, much has been done about shellfish allergens, but available information about tropomyosin allergens is limited to those of Crustacea, (such as shrimp, lobster, crab) much remains to be investigated on the molluscs tropomyosin. In this study, the cDNA coding for *S. constricta* tropomyosin has been isolated and sequenced. The tropomyosin consist of 284 amino acid residues with a calculated molecular mass of 32.96 kDa, similar to the tropomyosin of *B. glabrata*, which was the top match when searched in the protein database by *S. constricta* tropomyosin peptide masses. Sequence alignments show that *S. constricta* tropomyosin has a 65–72% homology with other molluscs tropomyosin, for example, the identity between *S. constricta* tropomyosin and *Haliotis diversicolor* (GenBank accession no: [Q9GZ71](#)), *Septoteuthis lessoniana* (GenBank accession no: [BAE54430.1](#)) and *Helix aspersa* (GenBank accession no: [O97192](#)) was 72, 71, and 70% respectively. However, just as Fuller's report [4], tropomyosin from *S. constricta* only has a 54–57% homology with those from some crustaceans and other invertebrate, such as house dust mite (57%) and cockroach (54%).

The complete coding cDNA sequence of the *S. constricta* tropomyosin was cloned into the PET28a express vector and transformed into *E. coli* BL21(DE3) for expression. After induced with IPTG, high level of recombinant tropomyosin protein was gained. In order to confirm if the induced protein was the recombinant tropomyosin, MALDI-TOF-MS was used to analysis the expressed protein and the result showed that the peptide masses of the induced protein match to tropomyosin from *B. glabrata*, so the expressed protein can be considered as *S. constricta* tropomyosin.

In the immunoblot experiments, 4 out of 11 serum samples have specific IgE that reacted with the purified recombinant tropomyosin, and these four serum samples also have positive reaction with the protein extracts of *S. constricta*. This may indicate the similar IgE-binding capabilities of the natural tropomyosin and the recombinant tropomyosin. Though tropomyosin have been identified as major allergens in some mollusks and crustaceans [9–11,

23, 24], as to the *S. constricta* studied here, the sensitization to recombinant *S. constricta* tropomyosin was found in 36% of *S. constricta* allergic patients' sera, which indicated that tropomyosin was the minor allergens in *S. constricta*. This result was similar to the Asturias's report that in snail extracts, tropomyosin represented a minor allergen involved in invertebrate cross-reactivity [32].

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