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Original Article

Characterization, homology modeling and expression of the putative translationally controlled tumor protein gene from giant river prawn *Macrobrachium rosenbergii* (de Man)

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ABSTRACT

The translationally controlled tumor protein (TCTP) is a multifunctional protein that promotes cell growth and viability. The TCTP has been associated with shrimp immune response by high levels being detected during the onset of white spot syndrome virus (WSSV) infection. The present study characterized the putative TCTP gene from the giant river prawn *M. rosenbergii* and investigated a putative function of this protein in response to *Aeromonas hydrophila* injection. A full-length cDNA sequence was obtained by assembly of short reads of RNA sequences from a 454-transcriptome dataset of river prawns and was designated as putative *Mr*TCTP. The three-dimensional (3D) structure of *Mr*TCTP was constructed using a homology modeling approach to predict the functional properties of the protein. Expression of *Mr*TCTP was assessed in hepatopancreas tissues 96 h after injection using quantitative real-time polymerase chain reaction. The 704 bp full length cDNA of *Mr*TCTP contained an open reading frame of 507 bp encoding a polypeptide of 168 amino acid residues. The conserved signature sequences of TCTP, I45-D55 and F123–Y145 shared 61–92.3% similarity with the previously identified TCTP. The quality assessment and parameter validation indicated that the putative *Mr*TCTP modeled structure was reliable. Expression levels of *Mr*TCTP were significantly different ($p < 0.05$) between the control and infected prawns during 16–48 h. Transcript levels significantly increased 1.21-fold at 16 h to 2.26-fold at 48 h. The results suggested that the putative *Mr*TCTP may be involved in the anti-apoptotic mechanism of river prawn.

Introduction

The giant river prawn *Macrobrachium rosenbergii* is an economically important species as it contributes substantially to aquaculture production in Thailand (FAO, 2017). However, bacterial and viral diseases are major problems that reduce the production of prawn farming, particularly in post-larval production (New, 2003). Outbreaks of Nodavirus and Extra small virus have been reported to cause mass mortality of post-larvae (Yoganandhan et al., 2006). In addition, the prawns are also susceptible to several pathogenic bacteria, including *Aeromonas* spp., the common pathogens which are found to be associated with black spot necrosis or shell disease (Sung et al., 2000; Keysami et al., 2007; Sahoo et al., 2007). Understanding the principles of innate immune response in river prawn and the specific genes that play important roles during bacterial infection is one strategy to solve the disease problems, that is, through the development of immunostimulant proteins from responsive genes (Bangrak et al., 2004; Tonganunt et al., 2008). The translationally controlled tumor protein (TCTP), also known as histamine releasing factor is an evolutionarily conserved multifunctional protein that is found ubiquitously in eukaryotes from yeasts to higher animals and has been well studied in humans, though it was originally identified in mice as a tumor protein and subsequently recognized for its role in regulating the cell cycle (Gnanasekar et al., 2009) and cancer progression (Cans et al., 2003). The protein is vital for growth and development in animals by which homozygous mutant (TCTP−/−) mice become embryonically lethal (Chen et al., 2007). TCTP participates in numerous cellular processes to protect cells against stress

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conditions by acting as a molecular chaperone and as an antioxidant (Gnanasekar and Ramaswamy, 2007; Gnanasekar et al., 2009). TCTP also functions as a calcium-binding protein (Kim et al., 2000; Gnanasekar et al., 2002) and as an anti-apoptotic protein (Rho et al., 2011) to promote cell viability. TCTP is a hydrophilic protein of approximately 18–23 kDa that shares no sequence similarity with other known proteins (Chitpatima et al., 1988; Bommer et al., 1994). TCTP has been reported to play a critical role in the immune response of tiger shrimp (*Penaeus monodon*) infected with white spot syndrome virus (WSSV), the most dangerous and economically damaging disease in the shrimp farming industry (Bangrak et al., 2004; Tonganunt et al., 2008). Results from these studies suggest potential applications of TCTP to improve shrimp disease resistance. Subsequently, the structure and immune functions of TCTP genes have been widely investigated in other species of marine shrimp, including kuruma shrimp *Masupenaeus japonicus* (Chen et al., 2009), Chinese white shrimp *Fenneropenaeus chinensis* (Wang et al., 2009), Pacific white shrimp *Litopenaeus vannamei* (Wu et al., 2013) and Indian prawn *F. indicus* (Rajesh et al., 2010; Nayak et al., 2014). However, the immune functions of TCTP remain to be clearly elucidated in shrimp and prawns. The present study characterized TCTP cDNA of giant river prawn (*M. rosenbergii*) from its transcriptomic dataset and determined the three-dimensional (3D) structure to predict and to gain a better understanding of the functional properties of the TCTP protein. In addition, to investigate its role in immune response, gene expression was analyzed in juvenile prawns injected with *Aeromonas hydrophila*. The results of the study should provide the first insight into the molecular characterization and the 3D structure of TCTP which may help to better understand the function of TCTP in *M. rosenbergii*.

Materials and methods

Identification and sequence analysis of putative M. rosenbergii *TCTP*

A review on immune gene discovery in *Penaeus momondon* by Tassanakajon et al. (2013) was used as a reference to search for potential immune genes in prawns from the transcriptome dataset of *M. rosenbergii* established from previous unpublished data. Briefly, unigenes obtained from the assembly of short reads from RNA sequencing were evaluated for sequences of immune-relevant genes via gene ontology (GO) using Blast2GO (Conesa et al., 2005). Subsequently, the TCTP gene was identified through a BLAST homology search against the NCBI database (http://blast.ncbi.nlm.nih.gov). The open reading frame (ORF), the 5′ and 3′ untranslated regions (UTRs) of the cDNA and the amino acid sequence of this gene were analyzed using ORF Finder (Open Reading Frame Finder, http://www.ncbi.nl.nih.gov/gorf/gorf.html) and the Expasy Translate tool (http://web.expasy.org/translate/). Bioinformatics tools were applied to analyze and predict the protein structure and function of TCTP; SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP/) was used to detect signal peptides and to identify the predicted start of a translated sequence. Signal peptides, functional sites, and domains in the predicted amino acid sequences were predicted using the Simple Modular Architecture Research Tool (SMART) program (http://smart.embl-heidelberg.de). The full-length gene was designated as putative *Mr*TCTP (http://www.ebi.ac.uk/Tools/msa/clustalo/). The identity of amino acid sequences was evaluated using MatGAT (Matrix Global Alignment Tool) version 2.02 (http://bitincka.com/ledion/matgat/). The predicted protein sequences were aligned with various known TCTP sequences using the CLUSTAL OMEGA Multiple Sequence Alignment program (http://www.ebi.ac.uk/Tools/msa/clustalo/) and the structural alignment was generated at the ESPript server; http://espript.ibcp.fr/ESPript/ESPript (Gouet et al., 1999). For phylogenetic analysis, amino acid sequences of TCTP were aligned using the MEGA software version 6.06 (Tamura et al., 2013) with default settings and bootstrap test of 1000 replications. An unrooted phylogenetic tree was constructed using the neighbor-joining method based on the corresponding amino acid sequences of TCTP selected from various species available in the public database.

Structural modeling and evaluations of putative Mr*TCTP*

The BLAST program, protein-protein BLASTP module (https://blast.ncbi.nlm. nih.gov/Blast) was used to find homologous proteins against the Protein Data Bank (PDB) (http://www.rcsb.org). Suitable templates with the highest sequence identity were used to generate the 3D model structure of the putative *Mr*TCTP gene using the automated homology modeling server, SWISS-MODELWORKSPACE (Biasini et al., 2014). The nuclear magnetic resonance (NMR) structure of human TCTP, PDB accession number 2HR9 (Susini et al., 2008) and crystal structures of human TCTP (hTCTP), 3EBM (Dong et al., 2009), as well as TCTP from *Plasmodium knowlesi* 1TXJ (Vedadi et al., 2007) were selected as templates for further comparative modeling approaches. The model of *Mr*TCTP was then evaluated using several computational approaches. The stereochemistry of this model was examined using PROCHECK interactive server v.3.5 (http://services.mbi.ucla.edu/PROCHECK/) (Laskowski et al., 1993). The reasonable folding of the modeled putative *Mr*TCTP was evaluated along with the TCTP templates structures using the solvent accessible surface area (SASA) method. These SASA calculations were computed in Pymol v.0.99 (DeLano, 2002). All structural figures were visualized using Discovery Studio 3.5 (Accelrys Inc.; San Diego, CA, USA) and Pymol v.0.99 (DeLano, 2002).

Bacterial challenge

In total, 180 juvenile prawns aged 3 mth with an average body weight of 2.3 ± 0.8 g were obtained from a farm in Suphanburi province, Thailand and were divided into four groups which were stocked into four 60 L aquaria with 45 prawns per aquarium. Prawns in the first group served as a negative control. Prawns in group 2, group 3 and group 4 each represented a replication. The bacterium *A. hydrophila* strain AQAH03 was used in a challenge test. Prawns were intramuscularly injected with 1.2×10^6 CFU/mL of *A. hydrophila* (Lethal $Dose_{50}$, LD_{50} concentration at 48 h) at the third abdominal segment. To verify that challenged individuals died from bacterial infection and not from the injection procedure, 45 prawns were injected with 80 μL of 0.85% $NaCl_2$ and served as the negative control. During the course of infection, prawns were fed commercial pellets twice daily. After a bacterial challenge, all groups were observed every 2 h until 96 h post challenge to monitor mortality. For each replicate, hepatopancreas tissues were collected from three individuals at 0 h, 2 h, 6 h, 12 h, 16 h, 24 h, 48 h, 60 h, 72 h and 96 h, respectively. Samples were stored at −80 °C until total RNA was isolated.

Mr*TCTP expression by quantitative real time polymerase chain reaction*

Hepatopancreas tissues (approximately 50 mg each sample) of three prawns in each replicate of the challenged groups were pooled for RNA extraction using TRIzol reagent (Molecular Research Center, Inc.; Cincinnati, OH, USA) according to the manufacturer's instructions. The concentration and purity of RNA samples were determined using spectrophotometry with a

NanoDrop (NanoDrop Technologies; Wilmington, DE, USA). The final concentration was adjusted to 1 μg of total RNA and the first strand cDNA was synthesized using a RevertAid First Strand cDNA Synthesis Kit (Fermentas; Waltham, MA, USA) following the recommendation of the manufacturer, and all cDNA samples were kept at −20 °C until used. Relative expression levels of *Mr*TCTP in the hepatopancreas tissues of the control and the treated groups were measured using quantitative real time polymerase chain reaction (qPCR). The *M. rosenbergii* β-actin gene was used as an internal control and nuclease-free water was used as the negative control during all qPCR runs. The primer details of gene specific primer *Mr*TCTP and β-actin were: *Mr*TCTP F: TATGAGGTTGTC-GACGATGC and *Mr*TCTP R: GTTTCCAAAGCCAGTCTCCT and β-actin F: CTGGTACCACCTGATACTGT and β-actin R: GTCTCTGA-GATTCTAGCTCC. The real-time PCR was performed using the Brilliant II SYBR Green qPCR Master Mix (Stratagene; USA), following the manufacturer's protocol on the Mx3005P real-time PCR system (Stratagene; Santa Clara, CA, USA) equipped with analytical software version 4.0. The PCR reaction was performed in a total volume of 12.5 μL, containing 6.25 μL of 2 × SYBR Green qPCR Master Mix, 0.5 μL of each gene specific primer (5 μM), 0.5 μL of cDNA template and 4.75 μL of nuclease-free water. The PCR cycling conditions were: 95 °C for 10 min, followed by 40 cycles at 95 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min. Data were calculated using $2^{-\Delta\Delta Ct}$ methods (Livak and Schmittgen, 2001) and subjected to statistical analysis. The relative expression levels between challenged prawns at different time intervals were statistically tested using one-way analysis of variance followed by Duncan's new multiple range test. Results were considered significant if $p < 0.05$.

Results and discussion

Characterization of putative Mr*TCTP*

The full length cDNA of *Mr*TCTP was 704 bp, containing an ORF of 507 bp (from 62 to 568 nucleotides) encoding a polypeptide consisting of 168 amino acid residues with a calculated molecular weight of approximately 18.94 kDa and a theoretical isoelectric point of 4.48. It consisted of a 61 bp 5′ untranslated region (UTR) and a 3′ UTR of 136 bp. Signal P analysis revealed that this protein has no signal peptide. An N-glycosylation site from 34 to 37 (NTTV), three casein kinase II phosphorylation sites (CK2-phospho-sites) from 9 to 12 (SGDE), from 50 to 53 (SAEE), and from 64 to 67 (SGID), and a putative site for phosphorylation (SER9, 17, 50, 62, 64, 64, 98, THR103, and TYR18, 20, 28, 92, 145) were also present. Additionally, an ion response element (IRE) was found at the 5′ UTR of *Mr*TCTP. The deduced amino acid sequence of *M. rosenbergii* contained a characteristic domain of the TCTP (histamine-releasing factor) family together with the Mss4-like superfamily showing two conserved TCTP signatures, TCTP1 and TCTP2. The TCTP1 sequence was found from ILE45 to ASP55, including N-myristoylation (GANPS). This motif is attached to the proposed GTPase binding surface (Thaw et al., 2001; Venugopal, 2005), whereas TCTP2 was found from PHE123 toTYR145 in the amino acid sequence. The nucleotide and deduced amino acid sequences of the putative TCTP from *M. rosenbergii* are shown in Fig. 1. The completed sequence was deposited in GenBank with the accession number KY474044. Most eukaryotic TCTPs have TCTP1 with the mobile flexible loop and TCTP2 signatures (Thaw et al., 2001). As in other species, TCTP of *M. rosenbergii* also has a homologous microtubule binding

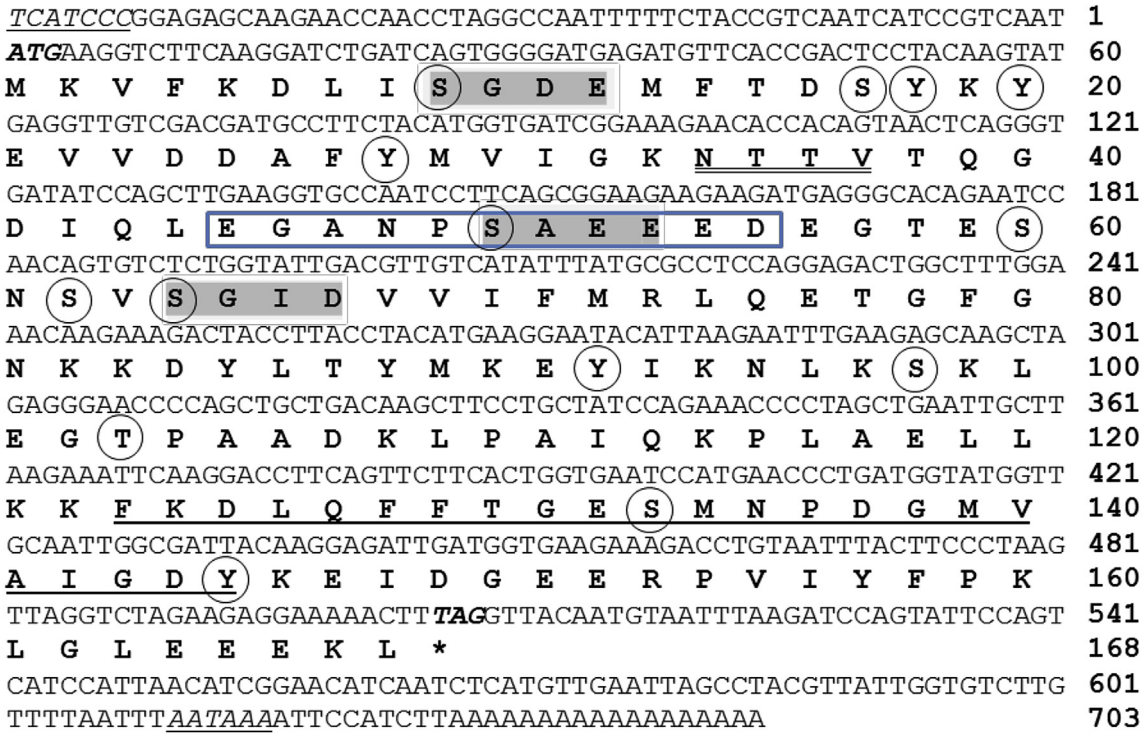

Fig. 1. Nucleotide and deduced amino acid sequences of a cDNA, putative *M. rosenbergii*, translationally controlled tumor protein (*Mr*TCTP). The putative amino acid sequence is shown in bold. The number indicates the position of the nucleotide and amino acids. ATG and TAG are start and stop codons. The putative ion response element (IRE) in the 5′ UTR is shown in italics and underlined. N-glycosylation site (NTTV) is double underlined. The conserved TCTP1 region is shown in a blue box and the TCTP 2 region is underlined. N-myristoylation (GANPS) is shown by a blue box and underlined. The circle represents the putative site for phosphorylation sites. Three CK2-phospho-sites (casein kinase II phosphorylation site) are in gray shading. The polyadenylation signal sequence is underlined. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

domain (Gachet et al., 1999) and a calcium binding domain (Kim et al., 2000) as shown in Fig. 2. Moreover, the BLASTX search revealed that *M. rosenbergii* TCTP exhibits a high sequence homology with TCTP obtained from the Subphylum Crustacea, including *Eriocheir sinensis*, *Marsupenaeus japonicas* and *F. chinensis* at 2E-98, 1E-93 and 3E-92, respectively.

Secondary structure, sequence alignment and phylogenetic tree analyses of putative Mr*TCTP*

According to the structure of human TCTP, PDB code: 2HR9 (Susini et al., 2008), the overall folding of *Mr*TCTP consisted of nine β-sheets and three α-helices connected to a protein complex (Fig. 2A). Multiple alignments of *Mr*TCTP amino acid sequences and 16 TCTP sequences from other species showed that *Mr*TCTP shared the highest sequence similarity with *E. sinensis* (84%), followed by *M. japonicus* (79%), *P. monodon* (79%), and *F. chinensis* (79%), indicating that the amino acid sequence of TCTPs is highly conserved in eukaryotic organisms (Fig. 2A). The phylogenetic tree showed that the putative TCTP sequences from *M. rosenbergii* and Chinese mitten crab (*E. sinensis*) were clustered in the same node, with a 75% bootstrap value (Fig. 2B). Representatives from the penaeid family were clustered in a separate clade with a 100% bootstrap value, suggesting an evolutionary conservation of TCTP within the penaeid family. Moreover, the putative *Mr*TCTP sequence was clearly separated from mammals and fish. The phylogenetic analysis confirmed the taxonomic relationships between *M. rosenbergii* and other species of crustaceans.

Comparative modeling and protein structure analyses of Mr*TCTP*

In the template selection process, the following criteria were considered, high structural identity, lowest e-value and low number of gaps. Among the protein homology searching hits, *Mr*TCTP shared a homologous sequence with seven protein structures from the protein data bank (PDB) with more than 30% sequence identity, namely 2HR9 (Feng et al., 2007), 3EBM (Dong et al., 2009), 1YZ1 (Susini et al., 2008), 1TXJ (Vedadi et al., 2007), 3P3K (Eichhorn et al., 2013), 2LOY (Lange et al., 2012) and 1H6Q (Thaw et al., 2001). According to Fig. 2A, the TCTP1 region is located on a flexible loop

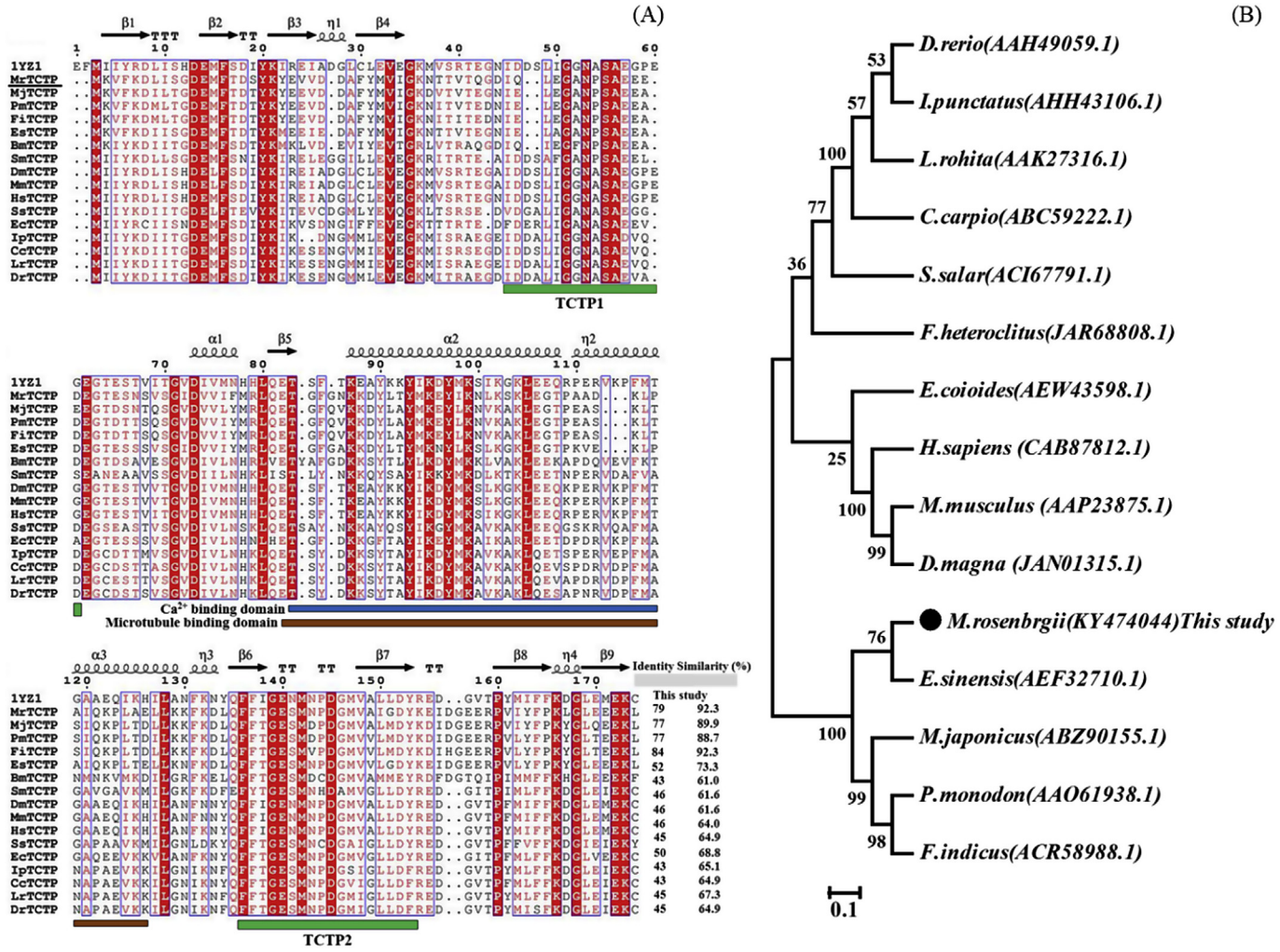

Fig. 2. Secondary structure, sequence alignment and phylogenetic tree analyses of putative *Mr*TCTP. (A) Secondary structure-based amino acid sequence alignment of putative translationally controlled tumor protein (TCTP) of giant river prawn and known TCTP from other species was performed by the CLUSTAL OMEGA program using human TCTP (2HR9) as a template. β-sheet by arrows, α-helices, η-helices are represented by spirals and strict β-turns are denoted TT, respectively. The TCTP1 and TCTP2 regions are marked on the figure by green bars. The microtubule-binding region and the Ca^{2+}-binding region are presented by a blue bar and an orange bar, respectively. Similar amino acids are highlighted in boxes and completely conserved residues are labeled by white lettering on a red background. (B) Phylogenetic tree of putative TCTP of giant river prawn with other species. The numbers are bootstrap values from 1000 replicates. The GenBank accession numbers are indicated within brackets. The bar represents the genetic distance. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

which presents as a similar sequence in various eukaryotic species. Of these eight PDB structures, four were solved using X-ray crystallography while the other four were solved using the NMR technique. As the flexible loop region highly fluctuates, it was observed in four NMR structures but was lacking in the four X-ray crystallographic structures. Of all the template structures from PDB, 2HR9 was selected according to the mentioned criteria (50.63% identity) with the presence of the TCTP1 region as well as the flexible loop.

The overlaid structures of human TCTP (2HR9) and putative *Mr*TCTP revealed that the core structure, consisting of a helical domain, β-stand domain, and a flexible loop, mostly matched, with only a few connecting loops being slightly different (Fig. 3A). Sequence alignment using the HHblits program (Remmert et al., 2012) between modeled putative *Mr*TCTP (M1-L168) and template human TCTP (2HR9, Q1-C173) indicated that both structures shared 50.63% identity and 44.0% similarity with a high range of distribution coverage (95%) as shown in Fig. 3B. In particular, the key conserved regions of TCTP, including TCTP1 and TCTP2 were almost identical. In the TCTP1 region, 3 out of 14 residues (21.42%) were identical, while 3 of the 14 residues (21.42%) were of similar types of amino acids and 3 of the 14 residues (21.42%) were deletion gaps. Moreover, the TCTP2 region presented greater complete sequence identity than TCTP1, in which 17 of 23 residues (73.91%) were identical. The entire structure and important sites/regions of the putative *Mr*TCTP are presented in Fig. 3C. The reliability and accuracy of the predicted model were evaluated using PROCHECK through the inspection of phi/psi angles of the Ramachandran plot. The results indicated that the structures of the putative *Mr*TCTP had good backbone conformational regularity for 87.6% of the residues in most favored regions and one amino acid was found in disallowed regions (0.7%). Solvent accessible surface area (SASA) is a computational tool which has been considered as one of the most important physical parameters for the prediction of physicochemical properties (Ali et al., 2014). This tool informs the reasonable folding of modeled putative *Mr*TCTP compared to its templates. The surface area of the putative *Mr*TCTP is presented in Fig. 3D. Understanding the protein surface properties is one of several approaches that may be useful for protein functional prediction (Burgoyne and Jackson, 2009). The quality assessment and

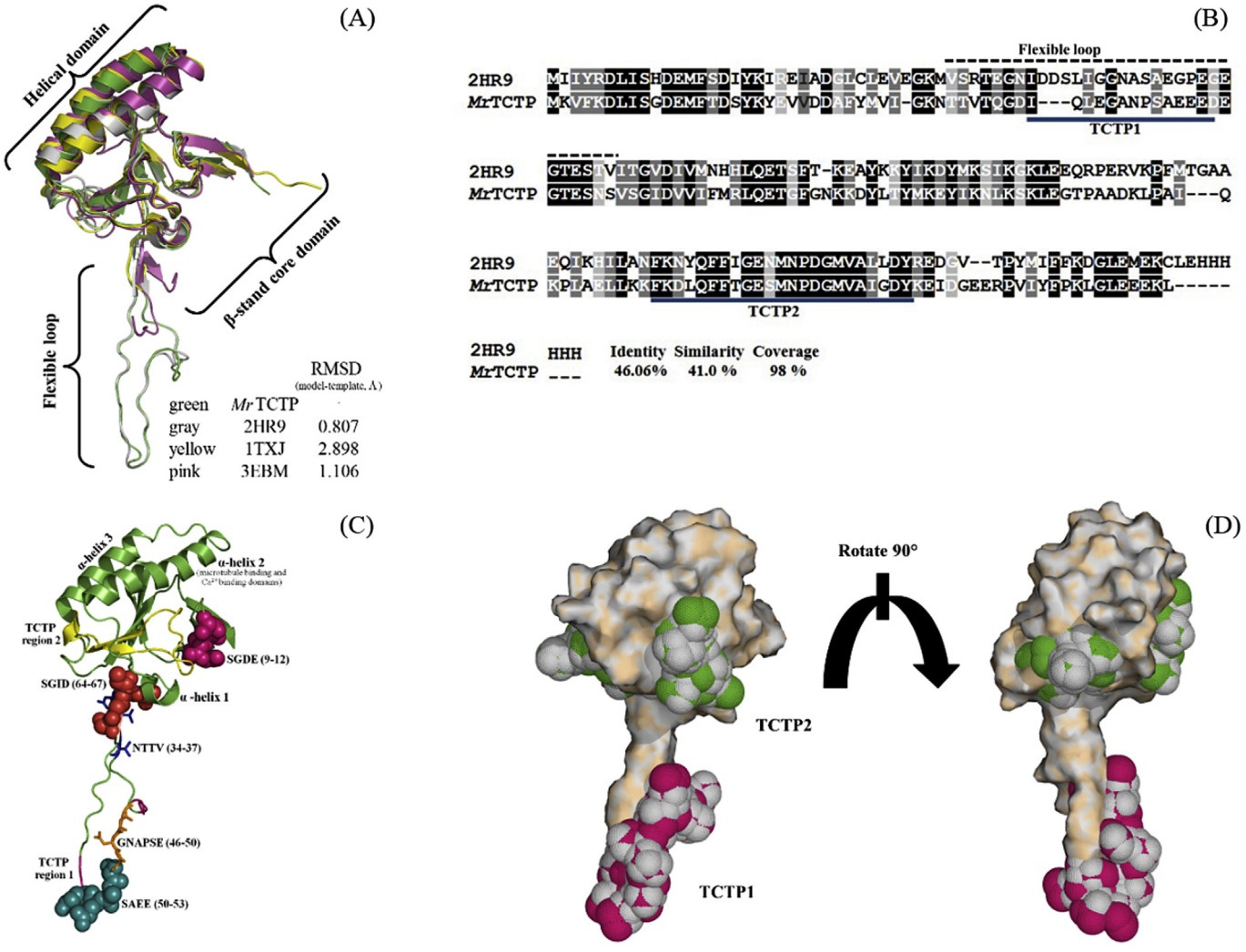

Fig. 3. A three-dimensional model of the putative *Mr*TCTP. (A) Superimposition of putative *Mr*TCTP model (green) is over 2HR9, 1TXJ and 3EBM represent in gray, yellow and pink, respectively and the root mean square deviation (RMSD) is shown in the figure. (B) Sequence alignments of putative *Mr*TCTP with 2HR9, identical residues are highlighted in black. (C) Homology model of putative *Mr*TCTP built based on the crystal structure of *H. sapiens* (2HR9 chain A) as a template. Predicted structure of putative *Mr*TCTP, N-glycosylation site (NTTV) is shown in the blue stick. The three CK2-phospho-sites; SAEE, SGDE and SGID are presented as cyan, pink and orange ball, respectively. N-muristoylation (GNASPE) is shown in dark yellow stick. Yellow ribbons represent the conserved TCTP1 region while TCTP2 region is shown in pink. (D) Surface representations of the TCTP1 and TCTP2 regions shown in pink and yellow, respectively. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

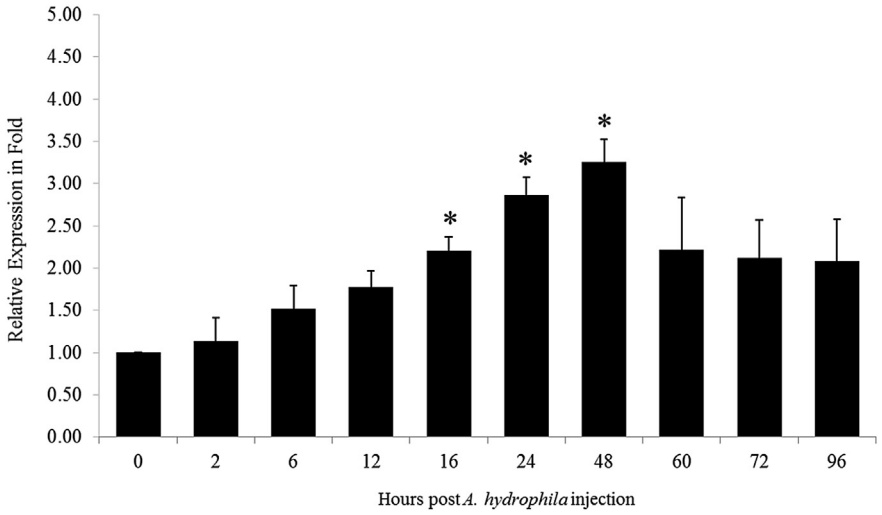

Fig. 4. Expression analysis of putative *Mr*TCTP transcript in hepatopancreas tissue of juvenile prawns after *A. hydrophila* injection. Relative expression level was normalized with β-actin taken as the reference. Results are presented as mean ± SD of fold changes. Asterisk indicates significant difference at $p < 0.05$ relative to control.

validation of parameters indicated that the putative *Mr*TCTP modeled structure was reliably folded.

Responses of putative Mr*TCTP to* A. hydrophila *injection*

The translationally controlled tumor protein (TCTP) is an important molecule involved in multiple biological processes, especially as an anti-apoptotic protein (Li et al., 2001; Liu et al., 2005; Graidist et al., 2006). Therefore, this part of the study focused on the expression profile of the *Mr*TCTP gene after *A. hydrophila* injection. The expression levels of *Mr*TCTP were significantly different between the control and infected groups (Fig. 4). The transcript levels significantly increased 1.21-fold, 1.87-fold, and 2.26-fold during 16 h, 24 h and 48 h, respectively, compared with the control group. After that, the transcripts decreased 1.22-fold, 1.21-fold, and 1.09-fold at 60 h, 72 h and

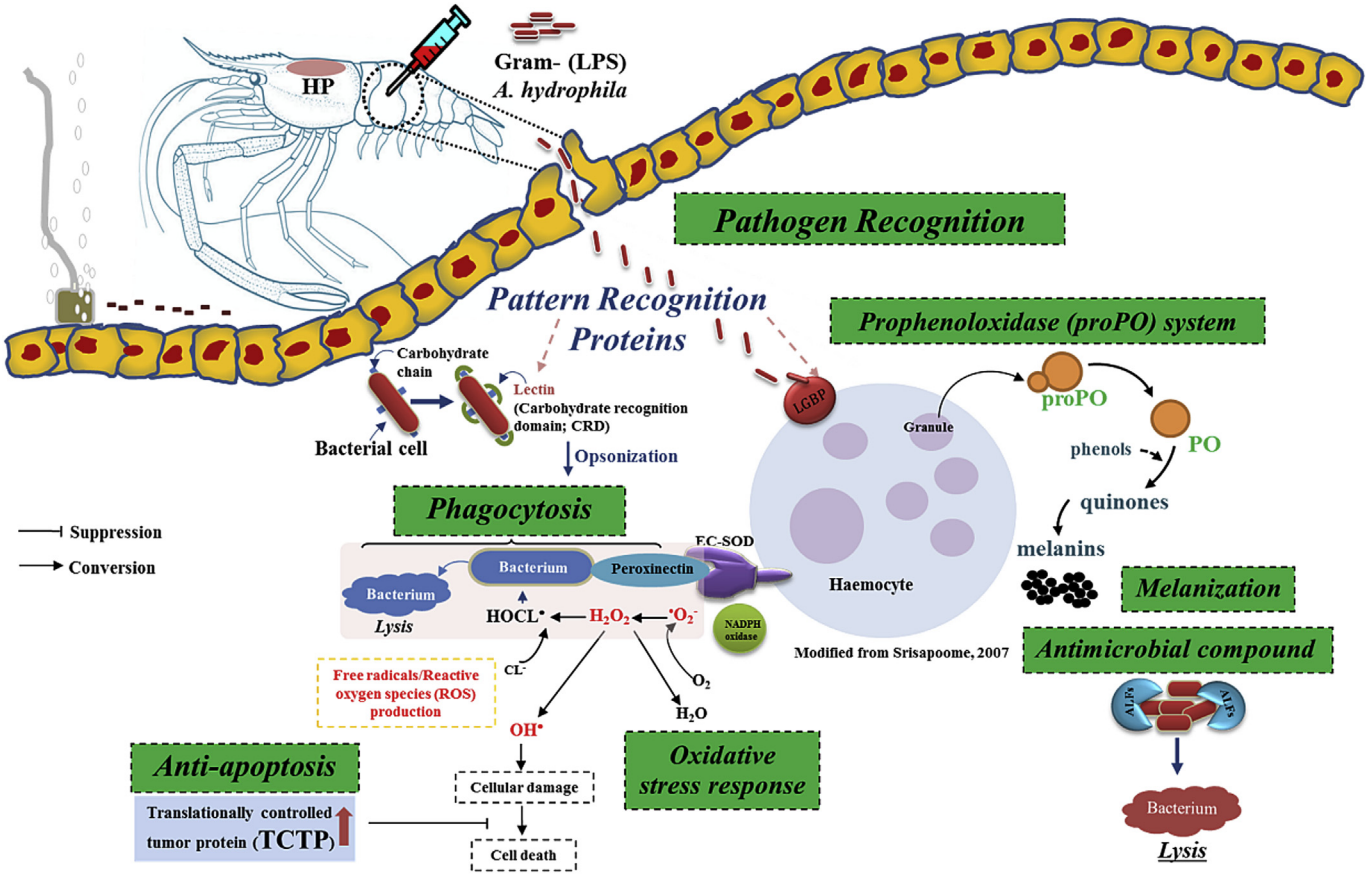

Fig. 5. Schematic model of prawn immune system.

96 h, respectively. The first mortalities were observed at 16 h post injection. Cumulative mortality gradually increased and reached 44% at 48 h. Individuals susceptible to *A. hydrophila* infection showed clear symptoms of disease including slow swimming and weak responses to stimulation. Subsequently, black shells and tail lesions were observed in these prawns. In contrast, prawns that survived to 60–96 h showed no signs of the disease. In the present study, the putative *Mr*TCTP gene may be involved in anti-apoptotic mechanisms in response to *A. hydrophila* infection by which the expression of TCTP was highly up-regulated during 16–48 h and decreased to a non-significant level compared to the control from 60 h to 96 h. A similar pattern of TCTP gene expression was observed in post larvae (PL14) of *P. monodon* after bath challenge with *Vibrio harveyi* D3 at 48 h when the expression level of TCTP increased 6.47-fold (Nayak et al., 2010). In addition, lethargic behavior and mortality were observed in *F. indicus* PL (Nayak et al., 2014). The results from these two studies suggested that TCTP plays a critical role in the survival of both shrimp species following *V. harveyi* infection through anti-apoptotic mechanisms. Similarly, Li et al. (2001) described the finding of human TCTP (or newly named fortilin) as a novel anti-apoptotic protein involved in cell survival. In another study, Bangrak et al. (2004) reported that the levels of TCTP expression decreased near the time of death in *P. monodon*, which showed acute systemic illness of WSSV-infection. The loss of TCTP from the hemocytes of shrimp may lead to the death of hemocyte cells and the loss of host defense, allowing propagation of the virus and resulting in the death of the shrimp. This hypothesis was supported by the study of Tonganunt et al. (2008) that tested recombinant TCTP (rFortilin) in WSSV-infected shrimp, showing 80–100% survival with very low copy numbers of WSSV using PCR. Their results suggested that the Fortilin/TCTP decreases viral infection probably by inhibiting viral replication. Because TCTP is a multifunctional protein, it is difficult to determine a specific role for TCTP during viral or bacterial infection. However, TCTP has been reported to be involved in apoptosis or programmed cell death by eliminating aberrant cells created by DNA damage or those infected by viral pathogens (Roulston et al., 1999). Wei et al. (2012) showed that TCTP might function as an antioxidant protein in marine fish where expression of TCTP was induced by hydrogen peroxide (H_2O_2) in the liver. Based on current information, the defense mechanism with a putative function of putative TCTP in *M. rosenbergii* can be summarized in Fig. 5. In this figure, TCTP performs anti-apoptosis functions by protecting host cell damage from free radicals produced by phagocytosis.

In conclusion, a full-length cDNA of TCTP was characterized, identified from the constructed cDNA library of the hepatopancreas of giant river prawn using 454 pyrosequencing technology. The homology model of putative *Mr*TCTP and its template, NMR structure of human TCTP (2HR9) shared two key conserved regions: TCTP region 1 and TCTP region 2. Validation of the proposed structure revealed that 87.6% or 127 of 168 residues were oriented in the most favored regions of the Ramachandran plot. The model was considered reliable for further *in silico* study. The current study contributes to basic knowledge of the putative *Mr*TCTP gene in response to *A. hydrophila* infection which can be useful for further research to understand the function of the TCTP protein. Molecular dynamic simulations can be applied to examine the stability of this model in solution system, mimicking natural conditions. Biological activities of putative *Mr*TCTP should be examined using western blot analysis and RNA interference to confirm the proposed model and cDNA results in future work. The information is useful for developing a disease control strategy to improve disease resistance in giant freshwater prawn.

Conflict of interest

The authors declare that there are no conflicts of interest.

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