Original Article

Biological activity of *Penaeus monodon* GILT in shrimp pathogen protection

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Abstract

Gamma-interferon-inducible lysosomal thiol reductase (GILT) contains a CXXC active site motif that possesses thiol reductase activity by catalyzing the disulfide bond reduction of exogenous antigens. Mutating the active site of human GILT to change the cysteine residues to serine residues eliminates this property. Our previous study reported that *Penaeus monodon* GILT (PmGILT) contained a CXXS active site motif. Therefore, we assessed the enzymatic activity of PmGILT and demonstrated that it displayed identical thiol reductase activity at an acidic pH. In addition, the biological activity of PmGILT against shrimp pathogens, including white spot syndrome virus (WSSV) and Gram-negative bacteria, was investigated. The neutralization of WSSV with PmGILT indicated the inhibition of WSSV invasion into shrimp hemocyte cells. Moreover, the relative percentage survival of shrimp injected with PmGILT-treated virus solution was 75%. Finally, the antimicrobial activity of PmGILT was confirmed by the growth inhibition of *Vibrio harveyi*. These results establish the role of PmGILT in the inhibition of the virulence of two major shrimp pathogens.

Keywords: shrimp (*Penaeus monodon*), gamma-interferon-inducible lysosomal thiol reductase (GILT), enzymatic activity, neutralization, antimicrobial assay

1. Introduction

In shrimp aquaculture, approximately 80% of the global commercial shrimp production loss occurs in Asia. Most of the production loss is caused by only two pathogen groups: viruses and bacteria. Viral and bacterial pathogens cause approximately 60% and 20% of disease losses in shrimp aquaculture, respectively (Flegel, 2012). White spot syndrome virus (WSSV) and *Vibrio* species are major pathogens that rapidly induce high levels of mortality in shrimp cultivation (Flegel, 2006; Longyant *et al.*, 2008). Therefore, finding therapies that protect shrimp from viral and bacterial pathogens is important.

In vertebrates, gamma-interferon-inducible lysosomal thiol reductase (GILT) has been identified as a unique thiol reductase that is involved in the MHC class II-restricted antigen processing and presentation pathway, which requires protein denaturation and the reduction of intra- and interchain disulfide bonds before lysosomal proteolysis (Arunachalam *et al.*, 1998; Collins *et al.*, 1991). GILT plays a key role in this step by catalyzing the reduction of disulfide bonds in exogenous antigens, thus facilitating the unfolding of the native protein antigen and simplifying the further cleavage of the antigen protein by cellular proteases (Arunachalam *et al.*, 2000; Li *et al.*, 2002; Maric *et al.*, 2001). Following antigen presentation, extracellular pathogens may be neutralized and cell debris resulting from bacterial infection may be cleared (Lackman & Cresswell, 2006). Moreover, additional functions of GILT have been reported. For example, GILT has a negative effect on T cell activation, as GILT-deficient T cells display more robust proliferative responses and cytotoxic activity than wild-type T cells (Barjaktarevic′ *et al.*, 2006). GILT is also associated with the regulation of fibroblast proliferation. The absence of GILT reduces the expression and activity of the mitochondrial manganese superoxide dismutase (SOD2) protein, which promotes fibroblast proliferation, by decreas-

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ing its stability (Bogunovic *et al.*, 2008). Furthermore, GILT is involved in the regulation of cellular redox homeostasis, as GILT-deficient fibroblasts exhibit decreased levels of the antioxidant glutathione, leading to a redox imbalance in the cytosol and the induction of autophagy (Chiang & Maric, 2011).

GILT is a soluble glycoprotein that is synthesized as a catalytically active 35-kDa precursor enzyme. Following delivery into the endosomal/lysosomal system via the mannose 6-phosphate receptor, N- and C-terminal propeptides are cleaved to generate the 30-kDa mature form of GILT. The precursor and mature forms of GILT are capable of catalyzing disulfide bond reduction in acidic conditions (optimum pH 4.5–5.5) both *in vivo* and *in vitro* (Arunachalam *et al.*, 2000). GILT is constitutively expressed in antigen-presenting cells (APCs) and is induced by interferon-ã (IFN-ã) in various cell types, such as fibroblasts, endothelial cells and keratinocytes (Arunachalam *et al.*, 2000; Luster *et al.*, 1988; Maric *et al.*, 2001; Phan *et al.*, 2001). GILT cDNA has been cloned and characterized in a variety of species. The major deduced GILT proteins contain a signature $CQHGX_2ECX_2NX_4C$ sequence, an active site CXXC motif, an Asn-linked glycosylation site and 10-11 conserved cysteines (Dan *et al.*, 2007; Dan *et al.*, 2008; De Zoysa & Lee, 2007; Cui *et al.*, 2011; Liu *et al.*, 2007; Phan *et al.*, 2001; Zhang *et al.*, 2010). The active site CXXC motif is similar to the WCGH/PCK motif in the thioredoxin family that acts as a catalyst for disulfide bond reduction (Phan *et al.*, 2000). However, unlike GILT proteins from other species, shrimp GILT contains a CXXS active site motif (Kongton *et al.*, 2011). The functional characterization of the active site CXXS motif is limited.

Our previous study reported that *Penaeus monodon* GILT (PmGILT) is involved in the shrimp immune response against viral and bacterial infections. The mRNA expression of *PmGILT* was highly upregulated in WSSV- and LPS-challenged shrimp. Nevertheless, the biological activity of PmGILT containing a CXXS active site motif is unknown. In this study, the enzymatic activity of PmGILT was assessed, and the biological activity of PmGILT against virus and bacteria was then observed by antiviral and antimicrobial assays. Notably, our results demonstrate that the recombinant PmGILT protein possessed thiol reductase activity and inhibited the virulence of shrimp pathogens at an acidic pH. From these results, we can infer that the PmGILT may protect against shrimp pathogens by catalyzing the disulfide bond reduction of pathogen antigens.

2. Materials and Methods

2.1 Shrimp culture

Healthy shrimp (*Litopenaeus vannamei*) weighing 10-15 g were obtained from a culture farm in Songkhla, Thailand. Before experimental use, five shrimps were randomly selected and tested for WSSV infection by PCR (Lo *et al.*, 1996). The shrimp tested negative were maintained in a continuously aerated 50-L aquarium tank. All experiments were performed in diluted natural seawater at 15 ppt salinity.

2.2 Preparation of WSSV stock

The WSSV inoculum was prepared as previously reported by Nupan *et al.* (2011). The gills, heart and lymphoid organ of a WSSV-infected shrimp were homogenized in K-199 medium (1% W/V M199, 1.88 M NaCl, 0.06 M $CaCl_2 \cdot 2H_2O$, 0.1 M L-glutamine, 9.14 mM Hepes and a 10% (v/v) salt solution consisting of 0.05 M KCl, 0.12 M $MgSO_4 \cdot 7H_2O$, 0.16 M $MgCl_2 \cdot 6H_2O$ and 3.2 mM $NaH_4PO_4 \cdot 2H_2O$, pH 7.3-7.6) at 1:2 (w/v) using a sterile homogenizer. The suspension was centrifuged at 3000 rpm for 20 min and again at 8000 rpm for 30 min at 4°C. The supernatant was then filtered through a 0.45-µm Minisart® filter (Sartorius Stedim) and stored at -70°C until use. Quantification of WSSV copy number was measured by real-time PCR using *WSSV-ie1* gene as a reference gene.

2.3 Construction of PmGILT expression vector and site-directed mutagenesis

The full-length cDNA of the *PmGILT* gene was obtained as previously described (Kongton *et al.*, 2011). The *PmGILT* cDNA was cloned into the *Hin*dIII/*Eco*RI restriction sites of the pDhsp-V5/His expression vector (provided by Chu-Fang Lo, Department of Zoology, National Taiwan University) and the *Bam*HI/*Hin*dIII restriction sites of the pET-28a(+) expression vector (Novagen) to construct pDhsp_GILT and pET_GILT, respectively.

To evaluate the role of the CXXS active site motif, site-directed mutagenesis of the *PmGILT* gene was performed using a QuikChange® Lightning Site-Directed Mutagenesis Kit (Stratagene) with pET_GILT as a template. The PCR primers that were used for the mutagenesis of Ser to Cys at the active site motif were as follows: S15C-1 (5'-CCCTGTGT CCTTACTGCATCGACTTCGTG-3') and S15C-2 (5'-CACGA AGTCGATGCAGTAAGGACACAGGG-3'). The mutant was named pET_mGILT and was confirmed by DNA sequencing.

2.4 Expression and purification of recombinant PmGILT and mPmGILT proteins

The pET_GILT or pET_mGILT recombinant plasmids were transformed into competent *E. coli* BL21 (DE3) cells to produce His-PmGILT or His-mPmGILT fusion proteins, respectively. The expression of the recombinant proteins was induced by culturing the bacterial cells with 0.1 mM isopropyl-beta-D-thiogalactopyranoside (IPTG) at 25°C for 5 h. The soluble recombinant proteins were collected as described by Cao *et al.* (2005) and were purified using a HisTrap FF column (GE Healthcare) with the AKTAprime plus purification system (GE Healthcare). After purification, the recombinant proteins were dialyzed in acetate buffer, pH 5.5 (100 mM sodium chloride, 50 mM sodium acetate pH 5.5, 0.1% Triton-

X-100). The proteins were determined by SDS-PAGE and Western blot using an anti-His$_6$ tag antibody.

2.5 GILT activity assay

The GILT activity assay was performed as previously described by Lackman *et al.* (2007). Briefly, the purified recombinant proteins were preactivated with 25 µM DTT in sodium acetate buffer, pH 5.5 (0.1% Triton X-100, 100 mM NaCl, 50 mM sodium acetate, pH 5.5) for 10 min at room temperature. Subsequently, the preactivated proteins were incubated with 5 µM BODIPY FL L-cystine (Invitrogen) for 1 h at 37°C. The reactions were quenched by adding 1 M Tris, and fluorescence was measured immediately using a SpectraMax M5 microplate reader (Molecular Devices) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

2.6 *In vitro* WSSV neutralization assay

The hemolymph was removed from the ventral sinus of healthy shrimp using 27-gauge syringe and mixed with K-199 medium containing L-cysteine anticoagulant in ratio 1:1. The hemocytes were then collected by centrifugation for 2 min at 6500 rpm and 4°C and resuspended in K-199 medium for incubation with the PmGILT and mPmGILT recombinant proteins.

Next, 6.22×10^8 copies of WSSV inoculum were incubated for 1 h at 25°C with each of the purified recombinant proteins diluted in sodium acetate buffer, pH 5.5, or with BSA in sodium acetate buffer as a control. After this incubation, 3.0×10^6 hemocytes were incubated for 1 h at 28°C with identical volumes of each of the mixtures of proteins and WSSV. Subsequently, the mixtures were centrifuged for 2 min at 6500 rpm and 4°C, washed gently six times with PBS to remove unbound viruses and then resuspended in PBS. The supernatant solution of each sample was used as a template for real-time PCR.

2.7 *In vivo* WSSV neutralization assay

First, the WSSV stock was titrated to determine the time-mortality relationship of the shrimp infection to the neutralizing dose that was used in this experiment.

For the neutralization assay, 9.7×10^3 copies of WSSV were incubated for 1 h at 25°C with 80 µg of the purified PmGILT or mPmGILT recombinant protein diluted in sodium acetate buffer, pH 5.5, per shrimp. Next, 100 µl of the mixtures was injected intramuscularly into the shrimp. Simultaneously, the negative and positive control groups were injected with sodium acetate buffer, pH 5.5, and 9.7×10^3 copies of WSSV diluted in sodium acetate buffer, pH 5.5, respectively. The experiment was performed in triplicate; fifteen shrimp were used for each group. The mortality of the shrimp was monitored daily for 15 days following injection. The relative percentage survival (RPS) of the shrimp was calculated using

the following formula: 1 - (the percent mortality of the test group/the percent mortality of the positive control group) x 100 (Zhu *et al.*, 2009).

Fifteen days after injection, the hearts of the surviving shrimp were homogenized in TN buffer (20 mM Tris-HCl, 0.4 M NaCl, pH 7.4) and centrifuged for 10 min at 8000 rpm and 4°C. The supernatant was collected by centrifugation for 5 min at 8000 rpm and 4°C and was used as a template for real-time PCR (Youtong *et al.*, 2011).

2.8 WSSV detection by quantitative real-time PCR

To determine the WSSV copy number, the samples were used as a template for real-time PCR with the following *WSSV-ie1* gene-specific primers: IE1-F (5'-GACTCTACAAA TCTCTTTGCCA-3') and IE1-R (5'-TGCTGATAAACTCTT GAAGGAA-3') (Nupan *et al.*, 2011). Real-time PCR was performed with the iQ™ SYBR® Green Super Mix (BIO-RAD) in an Mx3000P™ real-time detection system (Stratagene). *β-actin* was used as an internal control. The cycling conditions and the standard curves were generated as previously report (Youtong *et al.*, 2011). Statistical significance was determined by one-way ANOVA analysis (SPSS software, version 14.0). Values were considered significant at $P<0.05$.

2.9 Antimicrobial assay

The agar well diffusion assay was used to analyze anti-Gram-negative bacteria activity by screening with Luria Bertani (LB) agar containing 1.5% NaCl for *Vibrio harveyi* and Mueller Hinton (MH) agar for *Escherichia coli*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*. The experiment was repeated three times, and the results of the triplicate experiments were averaged.

The minimal inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) assays were used to test anti-*V. harveyi* activity. The MIC was determined using a liquid growth inhibition assay (Blond *et al.*, 2002).

3. Results

3.1 Production of the recombinant PmGILT and mPmGILT proteins in *E. coli* BL21 (DE3)

The His-PmGILT and His-mPmGILT fusion proteins were induced in BL21 (DE3) competent *E. coli*. The induced cells were lysed, and the pellets were collected because the recombinant PmGILT and mPmGILT proteins were associated with the insoluble fractions. The inclusion bodies were dissolved in 8 M urea buffer and refolded by the dialysis method. The recombinant PmGILT and mPmGILT proteins were purified using a HisTrap FF column (GE Healthcare) by Ni^{2+} affinity chromatography and dialyzed in acetate buffer, pH 5.5, to prepare the recombinant proteins for the enzymatic activity assay. The recombinant PmGILT and mPmGILT proteins appeared as 24-kDa bands by 12% SDS-PAGE and

were detected by Western blot analysis using an anti-His$_6$ tag antibody (Figure 1).

3.2 GILT activity assay

Phan *et al.* (2000) reported that human GILT containing the CXXC active site motif catalyzes the disulfide bond reduction at an acidic pH. Mutation of human GILT at the CXXC active site motif to CXXS motif abolished this property. In contrary, *Arabidopsis thaliana* thioredoxin subgroup h III and mutant form of *Escherichia coli* DsbA protein containing the CXXS/A, show efficiency disulfide reductase activities (Serrato *et al.*, 2008; Wunderlich *et al.*, 1995). This result suggested the single active-site cysteine may be sufficient to catalyze disulfide bond reduction. The objective of this work is to investigate whether shrimp PmGILT can perform the thiol reductase activity by using the single cysteine (CXXS) and give the same activity with mPmGILT that contains CXXC. The enzymatic activity of PmGILT and mPmGILT was assessed in acidic condition (pH 5.5) by a fluorescence-based activity assay using BODIPY FL L-cystine as a substrate because the recombinant proteins were precipitated at their pI value (pI = 4.6). Figure 2 shows that the recombinant PmGILT and mPmGILT proteins catalyzed the disulfide bond reduction of BODPIY FL L-cystine in a concentration-dependent manner and exhibited high enzymatic activity identical to that of the positive control, 1 mM DTT (reducing agent).

3.3 *In vitro* WSSV neutralization assay

First, 6.22×10^8 copies of WSSV were incubated for 1 h at 25°C with 10, 20, 40 or 80 μg of the recombinant PmGILT or mPmGILT proteins diluted in sodium acetate buffer, pH 5.5, or with BSA in sodium acetate buffer as a control. Then, 3.0×10^6 hemocytes were incubated with the mixtures. The *WSSV-ie1* gene copy number was analyzed by real-time PCR. The copy number of the *WSSV-ie1* gene continuously decreased in a concentration-dependent manner in the hemocytes incubated with WSSV in the presence of either of the recombinant PmGILT or mPmGILT proteins, whereas the hemocytes incubated with the positive control viral mixture did not display reduced viral copy numbers (Figure 3). This experiment was repeated three times with similar results.

3.4 *In vivo* WSSV neutralization assay

Before being intramuscularly injected into shrimp, 9.7×10^3 copies of WSSV were incubated for 1 h with 80 μg/shrimp of the recombinant PmGILT or mPmGILT protein. The shrimp that were injected with PmGILT- or mPmGILT-treated virus solution showed 75% or 83% relative percentage survival (RPS), respectively, whereas the negative and positive controls that were injected with sodium acetate buffer, pH 5.5, and 9.7×10^3 copies of WSSV diluted in sodium acetate buffer, pH 5.5, showed 100% and 0% RPS, respec-
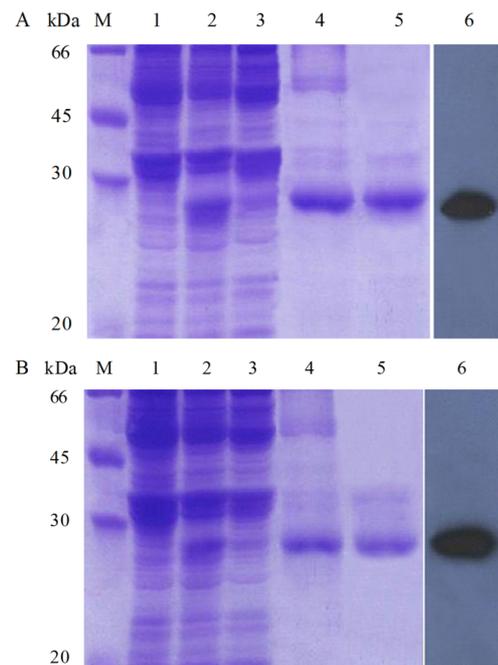
Figure 1. Coomassie Brilliant Blue-stained 12% SDS-PAGE showing the expression of the recombinant PmGILT (A) and mPmGILT (B) proteins in *E. coli* BL21 (DE3). Lane M, protein molecular weight markers; lanes 1 and 2, lysates of the recombinant plasmid-transformed bacteria before and after induction; lanes 3 and 4, supernatant and cell pellet of the lysed bacterial cells; lane 5, purified protein; and lane 6, Western blot analysis of the recombinant proteins using an anti-His$_6$ tag antibody.
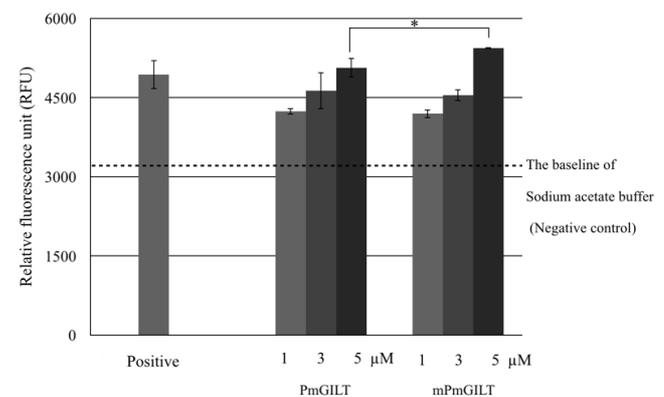
Figure 2. GILT activity assay of 1, 3 and 5 μM of recombinant PmGILT and mPmGILT proteins in sodium acetate buffer, pH 5.5 (0.1% Triton X-100, 100 mM NaCl, 50 mM sodium acetate buffer, pH 5.5). DTT (1 mM) was used as positive control (mean ± SD and n = 3). The significant difference (P < 0.05) is indicated by an asterisk (*).

tively (Figure 4). The experiment was performed in triplicate.

Fifteen days after the injection, the hearts of the surviving shrimp were used to determine the copy number of the *WSSV-ie1* gene by real-time PCR; heart is the main target organ of WSSV that support WSSV replication (Escobedo-
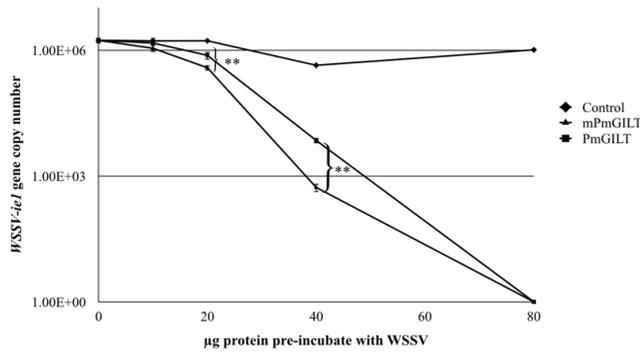
Figure 3. *In vitro* WSSV neutralization assay using the recombinant PmGILT or mPmGILT proteins (10, 20 40 and 80 µg) compared with the positive control (mean ± SD and n = 3). The significant difference (P < 0.01) is indicated by an asterisk (**).
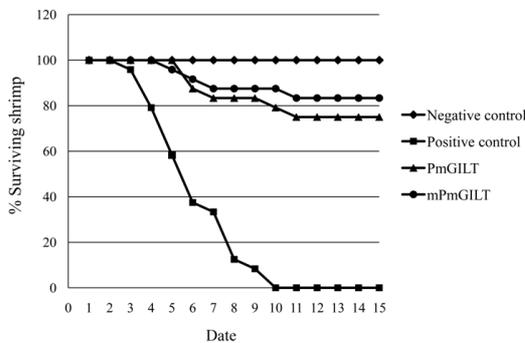
Figure 4. Percentage of surviving shrimp after injection with PmGILT- or mPmGILT-treated virus solution. The negative and positive controls were injected with sodium acetate buffer, pH 5.5 and 9.7×10^3 copies of WSSV diluted in sodium acetate buffer, pH 5.5, respectively.

Bonilla *et al.*, 2007; Lo *et al.*, 1997). The *WSSV-ie1* gene was not detected in the hearts of the shrimp injected with PmGILT or mPmGILT-treated virus solution. The positive control had 4.53×10^7 copies of *WSSV-ie1*/mg of heart tissue (Figure 5).

3.5 Antimicrobial assay

The *in vitro* antimicrobial activity of the recombinant PmGILT and mPmGILT proteins against Gram-negative bacteria was examined using an agar well diffusion assay by measuring the diameter of the growth inhibition zone. The growth inhibition zone of PmGILT against *V. harveyi* [13±1 mm] was larger than that against *E. coli* [8.3±0.29 mm], *E. cloacae* [7±0 mm] and *P. aeruginosa* [6.5±0 mm]. Identical results were observed for mPmGILT, as shown in Table 1. PmGILT and mPmGILT inhibit the growth of *V. harveyi* which give the similar size of inhibition zone when compared to Kanamycin (Rattanachuay *et al.*, 2010). Next, the MIC and MBC assays were used to tested anti-*V. harveyi* activity. The

recombinant PmGILT and mPmGILT proteins inhibited the growth of *V. harveyi* with MICs of 36.66 and 22.9 µg/ml and MBCs of 73.33 and 45.8 µg/ml, respectively (Table 2).

4. Discussion

Previously reported data indicated that PmGILT contains a CXXS active site motif resembling that found in the fruit fly GILT protein, NP_650287, but different from the CXXC active site motif found in the GILT proteins of various other

ND = not detected
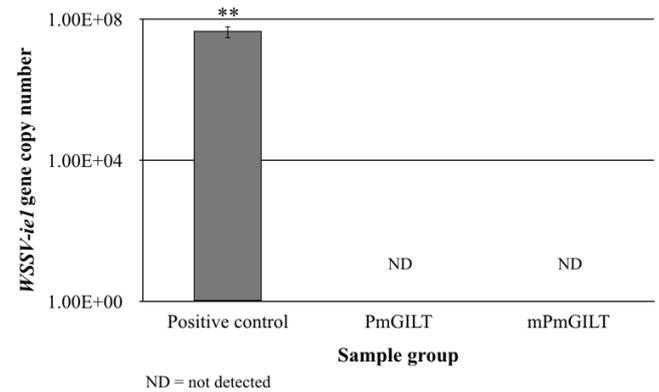
Figure 5. The copy number of the *WSSV-ie1* gene was analyzed in the hearts of the surviving shrimp after the injection of PmGILT- or mPmGILT-treated virus solution. Dead shrimp were used as a positive control (mean ± SD and n = 3). The significant difference (P < 0.01) is indicated by an asterisk (**).

Table 1. Antimicrobial activity of the recombinant PmGILT and mPmGILT proteins against Gram-negative bacteria using agar well diffusion assay

Test organisms	Zone of inhibition (mm)[a]	
	mPmGILT	PmGILT
Vibrio harveyi	14±1*	13±1*
Escherichia coli	8.3±0.29	8.3±0.29
Enterobacter cloacae	7±0	7±0
Pseudomonas aeruginasa	6.5±0	6.5±0

[a] The values for the zone of growth inhibition including the diameter of well (5 mm) are presented as the mean ± SD. The significant difference (P < 0.05) is indicated by an asterisk (*).

Table 2. MIC and MBC of the recombinant PmGILT and mPmGILT proteins against *V. harveyi*.

MIC (µg/ml)		MBC (µg/ml)	
mPmGILT	PmGILT	mPmGILT	PmGILT
22.9	36.66	45.8	73.33

organisms (human, AAH31020; large yellow croaker, DQ295788; amphioxus, AAQ83892; orange-spotted grouper, EF491185; abalone, DQ821495; pig, EF644197; pearl oyster, GQ480838; sheep, HM017967; South African clawed frog, JN202725) (Ai *et al.*, 2011; Arunachalam *et al.*, 2000; Cui *et al.*, 2011; Dan *et al.*, 2007; ; Dan *et al.*, 2008; De Zoysa & Lee, 2007; Liu *et al.*, 2007; Zhang *et al.*, 2010; Zheng & Chen, 2006). Several studies have demonstrated that the CXXC active site motif within the GILT protein catalyzes intra- and interchain disulfide bond reduction at an acidic pH (pH 4.5-5.5) (Ai *et al.*, 2011; Arunachalam *et al.*, 2000; Cui *et al.*, 2011; Phan *et al.*, 2001). The CXXC active site motif, N-terminal cysteine is proposed to initiate a nucleophilic attack on the target disulfide bond to generate the formation of an enzyme-substrate mixed disulfide intermediate and following the attack of the C-terminal cysteine to release the reduced substrate (Phan *et al.*, 2000). Mutating the second active-site cysteine of human GILT to serine or alanine abolished this activity by trapping the enzyme-substrate complex both *in vitro* and *in vivo* (Phan *et al.*, 2000). We therefore investigated the thiol reductase activity of PmGILT. We found that both PmGILT (CXXS) and mPmGILT (CXXC) catalyze the disulfide bond reduction of substrates at an acidic pH. Likewise, Wunderlich *et al.* (1995) reported that the mutation of the second active-site cysteine of the DsbA bacterial protein does not eliminate its thiol reductase activity and suggested that the single active-site cysteine is sufficient to catalyze the disulfide-interchange reaction.

It is well known that GILT promotes the processing of MHC class II-restricted antigens and the presentation of exogenous antigens from bacteria (Collins *et al.*, 1991; Li *et al.*, 2002; Thai *et al.*, 2004; Yewdell & Bennink, 1990). Moreover, GILT has been described as facilitating the MHC class I-restricted recognition of endogenous antigens derived from viruses. GILT-free mice display decreased viral antigen cross-presentation efficiency; for instance, the cross-presentation of herpes simplex virus 1 (HSV-1) antigens to CD8$^+$ T cells requires prior GILT-mediated reduction before transfer into the cytosol for proteasomal processing (Singh and Cresswell, 2010). In this study, we demonstrate through both *in vivo* and *in vitro* assays that PmGILT can neutralize the virulence of WSSV and that PmGILT possesses antibacterial activity against *V. harveyi*. It is most likely that PmGILT may function in damaging both viral and bacterial antigens. However, the actual mechanism by which GILT functions in the invertebrate innate immune response remains incompletely understood, and further investigation of the function of GILT in innate immunity is required.

Recently, several shrimp proteins possessing protective functions against bacterial or viral infections have been identified, such as fortilin, phagocytosis activating protein (PAP) and anti-lipopolysaccharide factors (ALF). The protective efficiency of these proteins against viral or bacterial pathogens was studied to find applications in shrimp disease treatment in aquaculture (Khimmakthong *et al.*, 2011; Ponprateep *et al.*, 2009; Tonganunt *et al.*, 2008). Our results suggest that the PmGILT functions in resistance to the major shrimp pathogens WSSV and *V. harveyi*. Therefore, PmGILT may be a potential treatment for shrimp aquaculture.

5. Abbreviations

GILT: Gamma-interferon-inducible lysosomal thiol reductase; PmGILT: *Penaeus monodon* GILT; mPmGILT: Mutant *Penaeus monodon* GILT; WSSV: White spot syndrome virus; RPS: Relative percentage survival; *V. harveyi*: *Vibrio harveyi*; APCs: Antigen-presenting cells; IFN-γ: Interferon-γ; LB: Luria Bertani media; MH: Mueller Hinton media; MIC: Minimal inhibitory concentration; MBC: Minimum bactericidal concentration.

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