Molecular cloning and mRNA expression of two peptidoglycan recognition protein (PGRP) genes from mollusk *Solen grandis*

Xiumei Wei, Jianmin Yang, Dinglong Yang, Jie Xu, Xiangquan Liu, Jialong Yang, Jinghui Fang, Hongjin Qiao

_Article info_

*Article history:* Received 16 September 2011
Received in revised form 11 November 2011
Accepted 11 November 2011
Available online 20 November 2011

**Keywords:**
- *Solen grandis*
- PGRP
- Innate immunity
- Pattern recognition receptor
- Real-time PCR

**Abstract**

Peptidoglycan recognition proteins (PGRPs) play crucial role in innate immunity for both invertebrates and vertebrates, owing to their prominent ability in detecting and eliminating invading bacteria. In the present study, two short PGRPs from mollusk *Solen grandis* (designated as SgPGRP-S1 and SgPGRP-S2) were identified, and their expression patterns, both in tissues and toward three PAMPs stimulation, were then characterized. The full-length cDNA of SgPGRP-S1 and SgPGRP-S2 was 1672 and 1285 bp, containing an open reading frame (ORF) of 813 and 426 bp, respectively, and deduced amino acid sequences showed high similarity to other members of PGRP superfamily. Both SgPGRP-S1 and SgPGRP-S2 encoded a PGRP domain. The motif of Zn$^{2+}$ binding sites and amidase catalytic sites were well conserved in SgPGRP-S1, but partially conserved in SgPGRP-S2. The two PGRPs exhibited different tissue expression pattern. SgPGRP-S1 was highly expressed in muscle and hepatopancreas, while SgPGRP-S2 was highly in gill and mantle. The mRNA expression of SgPGRP-S1 could be induced acutely by stimulation of PGN, and also moderately by $\beta$-1,3-glucan, but not by LPS, while expression of SgPGRP-S2 was significantly up-regulated ($P < 0.01$) when *S. grandis* was stimulated by all the three PAMPs, though the expression levels were relatively lower than SgPGRP-S1. Our results suggested SgPGRP-S1 and SgPGRP-S2 could serve as pattern recognition receptors (PRRs) involved in the immune recognition of *S. grandis*, and they might perform different functions in the immune defense against invaders.

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1. Introduction

Invertebrates lack an acquired immune system and their defense mechanisms against continuous threats from pathogens are based mainly on innate immune responses [1,2]. Among the numerous responses of innate immunity in invertebrates, discriminating self from harmful non-self mediated by pattern recognition receptors (PRRs) is the premier and crucial step [3]. At least six groups of PRRs have been identified from invertebrates [4], and they are believed to recognize different kinds of pathogen-associated molecular patterns (PAMPs) on pathogens. Among them, peptidoglycan recognition protein (PGRP) is the unique one to specifically recognize bacterial peptidoglycan (PGN), including both the Lys-type PGN from Gram-positive bacteria and mesodiaminopimelic Dap-type PGN from Gram-negative bacteria [5,6].

PGRP was firstly identified as a 19 kDa protein from hemolymph of *Bombyx mori*, which has affinity for PGN and the ability to trigger the prophenoloxidase cascade upon its binding to PGN [7]. Subsequently, PGRPs have been also identified from many other organisms [4,8–10], and they are characterized as conserved molecules in both invertebrates and vertebrates [11]. Thirteen different PGRP genes have been identified from *Drosophila*, and they are transcribed into at least 17 PGRP proteins [10]. According to the predicted structures and molecular weight, PGRPs identified are divided into three classes: short extracellular PGRPs (PGRP-S) of 20–25 kDa, intermediate PGRPs (PGRP-I) of 40–45 kDa with two predicted transmembrane domains, and long PGRPs (PGRP-L) of up to 90 kDa intracellular or membrane-spanning proteins [12]. The diversity of PGRPs in both category and structure suggests the multi-functions of PGRPs in innate immunity.

Recently many members of the PGRPs superfamily have been proved to play an emerging role in immune surveillance, for their wide involvement in innate immune responses. Most *Drosophila* PGRPs can serve as PRRs to recognize and bind bacterial PGN...
[7,8,13], and then to activate or inhibit the downstream immune responses, such as prophenoloxidase (proPO) system [7,14], Toll and IMD pathway [15–17], or JNK pathway [18–20]. In addition, as a result of the well conserved key motifs, including Zn2+ binding sites and amidase catalytic sites, some PGRPs can also function as N-acetylglucosamine-binding or N-acetylmuramyl-L-alanine amidases (NAMLLA) to cleave the lactylamide bond between muramic acid and the peptide chain in bacterial PGN [13,20–23], and exhibit bactericidal activity like T7 lysozyme [13,24,25]. Furthermore, several PGRPs such as PGRPs from seastar Asterias rubens and Drosophila are proved to serve as opsonins to induce agglutination or phagocytosis [21,26].

In view of the significant roles of PGRPs in the innate immune defense, study of PGRPs has also been performed in mollusk animals recently, though compared with that in arthropods and mammals, it is still in its infancy. Two short PGRPs, CIPGRP-S1 and AIPGRP, have been identified from scallop Chlamys farreeri and Argopecten irradians respectively, and both of them can be induced by the stimulation of PGN [12,27]. Besides CIPGRP-S1 is also proved to serve as an amidases eliminating the invading bacteria [12]. Another four short PGRP genes have also been cloned from Pacific oyster Crassostrea gigas, and they exhibit different tissue expression patterns [28,29]. Exploring novel members of molluscan PGRPs and revealing their potential roles in innate immunity, are essential for further understanding the mechanism of PGRP involved in mollusk immune system. In present study, two short PGRPs (SgPGRP-S1 and SgPGRP-S2) were identified from razor clam Solen grandis, and the distribution of their amidase sequences in different tissues as well as temporal expression response to stimulation of three typical PAMPs were characterized to unveil their possible participation in the immune recognition of mollusk animals.

2. Materials and methods

2.1. Razor clam

Naturally captured healthy razor clams S. grandis with average shell length of 85 mm, from Yantai, Shandong Province, China, were collected and maintained in aerated seawater at 20°C for a week before processing.

2.2. EST analysis and cloning of the full-length SgPGRP-S1 and SgPGRP-S2 cDNA

A cDNA library was constructed with the whole body of a razor clam, using the SMART cDNA Library Construction Kit (Clontech). Random sequencing of the library using M13 primer yielded 2038 successful sequencing reactions. BLAST analysis of the EST sequences revealed that two ESTs of 745 and 780 bp were highly similar to the known PGRP sequences. The corresponding clones were selected and completely sequenced using primer M13-F and M13-R (Table 1).

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence [5’–3’]</th>
</tr>
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<tbody>
<tr>
<td>Oligo (dT)-adaptor</td>
<td>GCCACACCCGGCTGCCAAGTACTACTCTCTT17</td>
</tr>
<tr>
<td>M13-F (forward)</td>
<td>CTAAAACACGCCCGCCGCA</td>
</tr>
<tr>
<td>M13-R (reverse)</td>
<td>CAGGAAACAGTATGACCC</td>
</tr>
<tr>
<td>SgPGRP-S1 RTF (forward)</td>
<td>TACCTGGGCAATGACCGAGGA</td>
</tr>
<tr>
<td>SgPGRP-S1 RTR (reverse)</td>
<td>CTCATATGACGGTGCCTTCAGCCAGGC</td>
</tr>
<tr>
<td>SgPGRP-S2 RTF (forward)</td>
<td>TGCAATGACGCTACCATACATAGGCTTG</td>
</tr>
<tr>
<td>SgPGRP-S2 RTR (reverse)</td>
<td>CGACACATCCTATAATGCTCTCAC</td>
</tr>
<tr>
<td>β-actin AF (forward)</td>
<td>TGTACGGGCAAACTGCGTTCGTC</td>
</tr>
<tr>
<td>β-actin AR (reverse)</td>
<td>CATCGTATTCCTGCTTGCGATCC</td>
</tr>
</tbody>
</table>

Table 1: Primers used in the present study.

2.3. Sequence analysis

The cDNA and deduced amino acid sequences of SgPGRP-S1 and SgPGRP-S2 were analyzed using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast) and the Expert Protein Analysis System (http://www.expasy.org/). The protein domains were predicted with the simple modular architecture research tool (SMART) version 4.0 (http://smart.embl-heidelberg.de/). Homology analysis was conducted using the Ident and Sim Analysis provided at http://www.bioinformatics.org/sms/. Multiple sequence alignment was performed with the ClustalW Multiple Alignment program [http://www.ebi.ac.uk/ clustalw/]. The presumed tertiary structures of SgPGRP-S1 and Drosophila PGRP-SC2 (CAD89187) were established using the SWISS-MODEL prediction algorithm (http://swissmodel.expasy.org) [30,31] and displayed by PyMOL version 0.97. A phylogenetic tree was constructed based on the deduced amino acid sequences of SgPGRP-S1, SgPGRP-S2 and other PGRPs by the neighbor-joining (NJ) algorithm using the MEGA4.1 software. To derive confidence value for the phylogeny analysis, bootstrap trials were replicated 1000 times.

2.4. Real-time PCR analysis of SgPGRP-S1 and SgPGRP-S2 mRNA expression in different tissues

The total RNA from hemocytes, gill, muscle, gonad, hepatopancreas and mantle were extracted from five adult razor clams as parallel samples using TRIzol reagent (Invitrogen). The first-strand synthesis was carried out based on Promega M-MLV RT Useage information using the DNase I (Promega)-treated total RNA as template and oligo(dT)-adaptor as primer (Table 1). The reaction mixtures were performed at 42°C for 1 h, and terminated by heating at 95°C for 5 min. The cDNA mix was diluted to 1:100 and stored at −80°C for subsequent SYBR Green fluorescent quantitative real-time PCR.

Two pairs of gene-specific primers for SgPGRP-S1 (SgPGRP-S1 RTF and RTR) and SgPGRP-S2 (SgPGRP-S2 RTF and RTR) (Table 1) were used to amplify products of 166 and 148 bp from cDNA, respectively. PCR products were sequenced to verify the specificity of RT-PCR. And β-actin gene was selected to normalize relative gene expression, for its convincible and statistically repeatable results. Two β-actin primers, AF and AR (Table 1), were used to amplify a 213 bp fragment as an internal control to verify the successful transcription and calibrate the cDNA template for correspond samples.

Real-time PCR amplification was carried out in an ABI 7300 Realtime Thermal Cycler according to the manual (Applied Biosystems). Dissociation curve analysis of amplification products was performed at the end of each PCR to confirm that only one PCR product was amplified and detected. After the PCR program, data were analyzed using ABI 7300 SDS software V2.0 (Applied Biosystems). To maintain consistency, the baseline was set automatically by the software. The 2−ΔΔCT method was used to analyze the expression level of SgPGRP-S1 and SgPGRP-S2 [32]. All data were given in terms of relative mRNA expressed as mean ± S.E. (N = 4).

2.5. Temporal expression analysis of SgPGRP-S1 and SgPGRP-S2 after PAMPs stimulation

Two hundred razor clams were employed for the PAMPs stimulation experiment. The razor clams were randomly divided into five groups and each group contained 40 individuals. Four groups received an injection of 50 μL phosphate buffered saline (PBS), 0.14 M sodium chloride, 3 mM potassium chloride, 8 mM disodium hydrogenphosphate dodecahydrate, 1.5 mM potassium phosphate...
monobasic, pH 7.4), LPS from Escherichia coli 0111:B4 (Sigma-Aldrich, 0.5 mg ml\(^{-1}\) in PBS), PGN from Staphylococcus aureus (Sigma-Aldrich, 0.8 mg ml\(^{-1}\) in PBS), glucan from Saccharomyces cerevisiae (Sigma-Aldrich, 1.0 mg ml\(^{-1}\) in PBS) via the muscle, respectively [33]. The group of untreated razor clams was employed as the blank. After the treatments, the razor clams were returned to water tanks and 5 individuals were randomly sampled at 3, 6, 12, 24 and 48 h post-injection.

The hemolymphs were collected, and centrifuged at 600 \(\times\) g for 10 min to harvest the hemocytes. RNA isolation, cDNA synthesis and RT-PCR analysis were carried out as described above. All data were given in terms of relative mRNA expressed as mean \(\pm\) SE (\(N = 4\)). The data were subjected to one-way analysis of variance (one-way ANOVA) followed by an unpaired, two-tailed \(t\)-test. Differences were considered significant at \(P < 0.05\) and extremely significant at \(P < 0.01\).

3. Results

3.1. Characteristic of the SgPGRP-S1 and SgPGRP-S2 full-length cDNA

Two ESTs from S. grandis cDNA library were found homologous to the previously identified PGRP genes. The clones corresponding to the ESTs were re-sequenced, and yielded two cDNA sequences of 1672 and 1285 bp, respectively. The CDNA sequences of SgPGRP-S1 and SgPGRP-S2 were deposited in GenBank under accession number JN642118 and JN642119.

The complete sequence of SgPGRP-S1 cDNA consisted of a 5\(^{'}\) terminal untranslated region (UTR) of 64 bp, a 3\(^{'}\) UTR of 795 bp with a poly (A) tail, and an open reading frame (ORF) of 813 bp (Fig. 1A). The ORF encoded a polypeptide of 270 amino acids with an isoelectric point of 7.82 and predicted molecular weight of 28.4 kD.
SMART analysis showed that the amino acids 1 (M) ~ 20 (H) encoded a signal peptide, while 21 (V) ~ 59 (C) and 60 (V) ~ 87 (V) encoded two repeat regions, and the amino acids 89 (A) ~ 125 (G) constituted a partial PGRP domain (Fig. 1A), which was usually found in PGRP superfamily members.

Compared with SgPGRP-S1, the full-length cDNA of SgPGRP-S2 consisted of a 5’ UTR of 67 bp, 3’ UTR of 792 bp with a poly (A) tail, and an ORF of 426 bp. The ORF encoded a polypeptide of 141 amino acids with an isoelectric point of 5.46 and predicted molecular weight of 14.7 kD. SMART analysis showed that the amino acids 1 (M) ~ 20 (H) encoded a signal peptide, while 21 (V) ~ 48 (G) and 60 (V) ~ 87 (V) encoded two repeat regions, and the amino acids 89 (A) ~ 125 (G) constituted a partial PGRP domain (Fig. 1B), compared with other PGRP domains.

### 3.2. Homologous and phylogenetic character of SgPGRP-S1 and SgPGRP-S2

BLAST analysis revealed that the deduced amino acid sequence of both SgPGRP-S1 and SgPGRP-S2 shared high similarity with PGRPs from other animals. SgPGRP-S1 was mostly homologous with PGRPs from Biomphalaria glabrata (ABG40829) and C. gigas (BAG31896), and the similarity was 57% and 56%, respectively. While SgPGRP-S2 shared high similarity with PGRPs from Saccoglossus kowalevskii (XP_002734591) and C. gigas (BAG31897), and the similarity was 55% and 49%, respectively. The two SgPGRPs shared 51% similarity with each other.

The two SgPGRPs and seven short PGRPs from other animals were used for multiple sequences alignment analysis. It was found that the C-terminal region of all the PGRPs compared was highly homologous and conserved, while the N-terminal was relatively diverse (Fig. 2). In SgPGRP-S1, the Zn\(^{2+}\) binding sites (H135, H244 and C252) and amidase catalytic sites (H135, Y170, H244, T250 and C252) were both well conserved (Fig. 2). However, the PGRP domain of SgPGRP-S2 was partial compared with that in other PGRPs, and only two amino acids (H115 and C123) of Zn\(^{2+}\) binding sites and three amino acids (H115, T121 and C123) of amidase catalytic sites were well conserved (Fig. 2).

A phylogenetic tree was constructed using neighbor-joining method with 1000 bootstrap test based on the multiple alignments of SgPGRP-S1, SgPGRP-S2 and PGRPs from other mollusk, Drosophila and human. In the phylogenetic tree, SgPGRP-S1 and...
SgPGRP-S2 were clustered together firstly, and then formed a sister group with PGPRs from mollusk *C. gigas* (Fig. 3).

### 3.3. The potential tertiary structures of SgPGRP-S1

The potential tertiary structures of SgPGRP-S1 and PGRP-SC2 from *D. melanogaster* (CAD89187) were established using the SWISS-MODEL prediction algorithm. Compared with *Drosophila* PGRP-SC2, tertiary structure and the secondary structure components of SgPGRP-S1 were well conserved, though the N-terminal random coil was shorter than that in *Drosophila* PGRP-SC2 (Fig. 4). Both of them adopted a typical PGRP structure consisting of five β-strands and five α-helices, and the two cysteine in α-helices 2 and random coil formed a disulfide bond (Fig. 4). The Zn²⁺ binding sites (H135, H244 and C252) and amidase catalytic sites (H135, Y170, H244, T250 and C252) in SgPGRP-S1 were both well conserved (Fig. 4a), which were H50-H159-C167 and H50-Y85-H159-T165-C167 in PGRP-SC2, respectively (Fig. 4b).

### 3.4. The mRNA expression profile of SgPGRP-S1 and SgPGRP-S2 in different tissues

The SYBR Green real-time PCR analysis was employed to study the mRNA expression of two PGRPs with β-actin as internal control. In the healthy razor clams, the mRNA transcripts of SgPGRP-S1 and SgPGRP-S2 were found to be constitutively expressed in a wide range of tissues with different levels, including mantle, gill, gonad, hemocyte, muscle, and hepatopancreas (Fig. 5). The SgPGRP-S1 mRNA was expressed with highest level in muscle and hepatopancreas, which was 45.3 and 38.7-fold compared with that in hemocyte, while lower level in gill and mantle, and relatively lowest in gonad and hemocyte (Fig. 5a). SgPGRP-S2 was with high level expressed in gill, mantle and hepatopancreas, and they were 692.9, 527.9 and 266.1-fold compared with that in muscle. While SgPGRP-S2 was with lower level expressed in hemocytes and gonad, and its expression level was lowest in muscle (Fig. 5b).
3.5. Temporal expression of SgPGRPs mRNA post PAMPs stimulation

The temporal expression of SgPGRP-S1 and SgPGRP-S2 mRNA in hemocytes were monitored after the razor clams were stimulated by three typical PAMPs. For SgPGRP-S1, LPS stimulation could not induce significant expression fluctuation (Fig. 6a). Compared with LPS, PGN stimulation induced more acute up-regulation of SgPGRP-S1. Transcripts of SgPGRP-S1 was significantly up-regulated to 14.3-fold ($P < 0.05$) of blank group at 3 h post PGN injection, while it then exhibited a burst of increase at 6 h after stimulation, which was 7471.0-fold ($P < 0.01$) higher than that observed in the blank group. After a sudden decrease at 12 h, the expression was up-regulated to 13.9-fold ($P < 0.01$) and 5.7-fold ($P < 0.01$) compared with blank group at 24 and 48 h, respectively (Fig. 6b). In the β-1,3-glucan stimulation group, expression of SgPGRP-S1 was also significantly ($P < 0.01$) up-regulated to 22.4, 11.9 and 89.7-fold compared with blank group at 12, 24 and 48 h post-injection, respectively (Fig. 6c). There was no significant change of SgPGRP-S1 expression in the control group treated with PBS (Fig. 6a).

Compared with SgPGRP-S1, SgPGRP-S2 exhibited more mitigation regulation patterns toward PAMPs stimulation. After LPS stimulation, expression of SgPGRP-S2 was significantly up-regulated, and it reached the maximal level at 6 h post stimulation, which was 4.1-fold ($P < 0.01$) compared with the blank group, and as time progressed it was significantly down-regulated to 0.1 and 0.3-fold ($P < 0.01$) of the blank level at 24 and 48 h post-injection (Fig. 6d). The expression pattern of SgPGRP-S2 after PGN stimulation was similar to SgPGRP-S1, and the expression level of SgPGRP-S2 reached the peak at 6 h (31.7-fold, $P < 0.01$) post stimulation, and then decreased sharply at 12 h, and subsequently up-regulated to 4.4-fold ($P < 0.05$) at 24 h after injection as the second time (Fig. 6e). In the β-1,3-glucan stimulation group, expression of SgPGRP-S2 was also significantly ($P < 0.01$) up-regulated, and the expression level at 12 h post-injection was 23.1-fold ($P < 0.01$) compared with blank group, and then the expression level decreased to the original level gradually (Fig. 6f). Throughout the process, the mRNA expression of SgPGRP-S2 in PBS group did not exhibit significant fluctuation (Fig. 6d–f).

4. Discussion

Accumulative evidences have suggested the significant roles of PGRPs in regulating the diverse immune responses of both Drosophila and vertebrates, such as pathogen recognition [7,8,13], degradation of PGN as bactericidal amidases [13,20–23], induction of phagocytosis [21,26], activation of prophenoloxidase (proPO) system [7,14], Toll and IMD pathway [15–17]. Though several PGRPs have been identified to date, the knowledge about mollusk PGRPs
The ORFs of SgPGRP-S1 and SgPGRP-S2 encoded a polypeptide of 270 and 141 amino acids, respectively, and the two SgPGRPs shared highly similarities with other short PGRPs. In the phylogenetic tree, both SgPGRP-S1 and SgPGRP-S2 kept a close evolutionary relationship with the other PGRPs, such as Drosophila PGRP-SC2, PGRP-LB, and PGRP-SB1 [13,23–25], and up to now all the molluscan PGRPs identified were short type, indicating the immune defense mechanism PGRPs mediated in mollusk might be in a different way with that in fruit fly and human. Compared with SgPGRP-S1 and other short PGRPs, the PGRP domain in SgPGRP-S2 was partial and it lacked of about 60 amino acids in the N-terminal of PGRP domain. More interestingly, except for the amino acids SgPGRP-S2 lacked, the ORFs of SgPGRP-S1 and SgPGRP-S2 were almost identical, though their 3′ UTRs were quite different with each other, and this might be as a result of alternative splicing, like the splicing mechanism of Drosophila PGRPs [10].

Some members of PGRP superfamily, such as mammalian PGLYRP-2 and Drosophila PGRP-SC2, PGRP-LB, and PGRP-SB1 [13,23–25], had amidase activity to degrade PGN by hydrolyzing the amide bond that linked the peptide units to the muramidic acid residues of the glycan strands [13,24,25], preventing excessive activation of the immune system by bacteria [18,20,25]. The PGRP-amidases were zinc-dependent enzymes, and structurally two motifs of the PGRP domain, the Zn²⁺ binding sites and amidase catalytic sites, were essential for their amidase activity [11]. In some other PGRPs, such as Drosophila PGRP-SA, -SD, -LE, or -LC, the absence of a critical cysteine residue within the PGRP domain abolished the enzymatic activity [19]. In present study, Zn²⁺ binding sites (H135, H244 and C252) and amidase catalytic sites (H135, Y170, H244, T250 and C252) required for amidase activity were both well conserved in SgPGRP-S1, but partially conserved in SgPGRP-S2. Furthermore, SgPGRP-S1 shared a similar tertiary structure with PGRP-SC2, an amidase-PGRP in Drosophila, especially on the amidase-related motifs. All these characteristics suggested SgPGRP-S1 might serve as an amidase involving in the elimination of PGN during the immune response against bacteria invading, but SgPGRP-S2 might be abolished the activity of amidase, owing to the absence of critical amino acids on the amidase-related motifs.

Unveiling the mRNA distribution pattern of PGRP in different tissues would benefit dissecting the potential functions they performed. Similar to other PGRPs genes, such as human PGLYRPs genes, zebrafish PGLYRPs genes and scallop PGRP-S1 [12,22,35], SgPGRP-S1 and SgPGRP-S2 exhibited a wide range of tissues distribution, and they expressed in hepatopancreas, muscle, gill, hemocyte, gonad, and mantle of the healthy razor clams. Considering razor clams completely expose themselves to the aquatic environment with large number of pathogenic microorganism, the wide distribution of SgPGRP-S1 and SgPGRP-S2 also suggested their important roles in immune defense of S. grandis against pathogenic microorganism infections. Interestingly, SgPGRP-S1 and SgPGRP-S2 exhibited different tissue expression pattern, indicating they might play different roles in the immune response. SgPGRP-S2, which dominantly expressed in gill and mantle, was suspected to be involved in the early recognition against microorganisms, while SgPGRP-S1 that mostly expressed in muscle and hepatopancreas, might mainly serve as an effective amidase involving in the elimination of bacterial PGN. However, further study is still needed to clarify the potential mechanism of these tissue expression patterns.

Most members of PGRP superfamily, for example scallop PGRP-S1, insect PGRP-S, -LB, -LC, and -LE, mouse and human PGRP-S, -L,
and [7,8,10,12,13], could recognize and bind PGN on the invading microorganism and then activate the downstream immune response. In present study, the expression of both SgPGRP-S1 and SgPGRP-S2 could be intensely induced by Gram-positive PGN injection, indicating that SgPGRP-S1 and SgPGRP-S2 was inducible acute-phase proteins that might be involved in the immune response against Gram-positive bacterial. Interestingly, though the expression level of SgPGRP-S1 was much higher than SgPGRP-S2 after PGN stimulation, the temporal expression pattern of SgPGRP-S1 and SgPGRP-S2 were nearly identical and both of them exhibited significant up-regulation for twice, which suggested SgPGRP-S1 and SgPGRP-S2 might not only be involved in the recognition of PGN in the acute response phase, but also functioned in the clearance of PGN after the acute response phase. In addition to PGN, other PAMPs such as LPS and glucan (or fungi) were also reported in the binding spectrum of PGRPs [9,12,36]. Similar results were also observed in present study, and both SgPGRP-S1 and SgPGRP-S2 could be induced by other PAMPs in addition to PGN. Expression of SgPGRP-S1 was significant up-regulated after stimulation of β-1, 3-glucan, while SgPGRP-S2 could be significantly induced by both LPS and β-1, 3-glucan. The result indicated SgPGRP-S1 and SgPGRP-S2 in S. grandis might serve as multiple-specific PRRs involving in the immune recognition toward different invading microbes.

Acknowledgments

We are grateful to all the laboratory members for their technical advice and helpful discussions. This research was supported by Program of Agriculture Thoroughbred Project, Shandong province, China to Dr. Xiangquan Liu.

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