Lipopolysaccharide and β-1,3-glucan-binding protein (LGBP) bind to seaweed polysaccharides and activate the prophenoloxidase system in white shrimp *Litopenaeus vannamei*

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**A B S T R A C T**

Lipopolysaccharide and β-1,3-glucan-binding protein (LGBP), important pattern recognition proteins (PRPs), recognize lipopolysaccharide (LPS) and β-1,3-glucan (βG), known as pathogen-associated molecular patterns (PAMPs), and subsequently trigger innate immunity. Several seaweed polysaccharides and seaweed extracts increase immune parameters and resistance to pathogens. Here, we constructed the expression vector pET28b-LvLGBP and transferred it into *Escherichia coli* BL21 (DE3) for protein expression and to produce the recombinant protein LGBP (rLvlGBP) in white shrimp *Litopenaeus vannamei*. We examined the binding of rLvlGBP with seaweed-derived polysaccharides including alginate, carrageenan, fucoidan, laminarin, *Gracilaria tenuistipitata* extract (GTE), and *Sargassum duplicatum* extract (SDE), and examined the phenoloxidase activity of shrimp haemocytes incubated with a mixture of rLvlGBP and each polysaccharide. We also examined the binding of rLvlGBP with LPS and βG, and the phenoloxidase activity of shrimp haemocytes incubated with a mixture of rLvlGBP and LPS (rLvlGBP-LPS) or a mixture of rLvlGBP and βG (rLvlGBP-βG). An ELISA binding assay indicated that rLvlGBP binds to LPS, βG, alginate, carrageenan, fucoidan, laminarin, GTE, and SDE with dissociation constants of 0.1138–0.1770 μM. Furthermore, our results also indicated that the phenoloxidase activity of shrimp haemocytes incubated with a mixture of rLvlGBP and LPS, βG, alginate, carrageenan, fucoidan, laminarin, GTE, and SDE significantly increased by 328%, 172%, 200%, 213%, 197%, 194%, 191%, and 197%, respectively compared to controls (cacodylate buffer). We conclude that LvlGBP functions as a PRP, recognizes and binds to LPS, βG, alginate, carrageenan, fucoidan, laminarin, GTE, and SDE, and subsequently leads to activating innate immunity in shrimp.

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**1. Introduction**

Shrimp rely on an innate immune response to defend against pathogens. Once foreign particles enter the haemocoel, shrimp engage an innate immune response that includes cellular and humoral reactions. This system is initiated via the recognition and binding of foreign molecules like lipopolysaccharide (LPS) and peptidoglycan (PG) derived from bacteria cell walls, and by β-1,3-glucan (βG) derived from fungi and yeast mycelia, which are known as pathogen-associated molecular patterns (PAMPs) of the host’s recognition molecules and are named pattern recognition proteins (PRPs) or receptors (PRPs) (Janeway and Medzhitov, 2002). Several types of PRPs have been reported in crustaceans including lipopolysaccharide and β-1,3-glucan binding protein (LGBP), β-1,3-glucan binding protein (βGBP), C-type lectin, tachylectin, masquerade-like protein, and peptidoglycan recognition protein (PGRP) (Lee and Söderhäll, 2002; Tassanakajon et al., 2013; Wang and Wang, 2013). LGBP and βGBP have been identified in white shrimp *Litopenaeus vannamei* (AY723297, EU102286), tiger shrimp *Penaeus monodon* (AF368168; Amparyup et al., 2012), and kuruma shrimp *Marsupenaeus japonicus* (AB162766, EU267001). Sequence analysis of amino acids between LGBP and βGBP share high similarity (99%) suggesting they are allelic variants of the same gene (Lin et al., 2008; Amparyup et al., 2012). LGBP and βGBP have two potential polysaccharide recognition motifs, polysaccharide binding motif (PSBM) and β-glucan recognition motif (βGRM), and a

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In crustacean, three types of haemocytes are identified based on cell size and degree of granularity (Tsing et al., 1989). These are the hyaline cells (HCs), semi-granular cells (SGCs), and granular cells (GCs). Semi-granular cells are characterized by a number of small granules, whereas granular cells are filled with large granules (Kitikiew et al., 2013). Both SGCs and GCs can be induced by foreign particle like LPS, βG, and PG to degranulate, and undergo exocytosis of granules to release several proteins like prophenoloxidase (proPO), peroxinectin, and prophenoloxidase activating enzyme (proppA) etc (Barracco et al., 1991; Soderhall et al., 1994; Jiravanichpaisal et al., 2006). The proPO system is carried out via the proteolytical conversion of proPO to phenoloxidase (PO) by active pPA in the presence of PAMP that subsequently results in the activation of innate immunity processes such as melanisation, releases of cytotoxic compounds, and encapsulation of pathogens (Cerenius et al., 2008).

In shrimp, once bacteria, fungi, or other foreign particles invade a host, βG or LGBP recognizes and then activates the serine proteinase cascade that activates the proPO system (Cerenius et al., 2008). For instance, purified βGGBP from Brazilian shrimp Farfantepenaeus paulensis and Litopenaeus schmitti has agglutination ability (Tsing et al., 1989). Seaweed polysaccharides (SPS), including curdlan, laminarin, LPS, and PG increase the PO activity of shrimp (Itami et al., 1998). In addition, seaweed-derived polysaccharides, such as alginate, carrageenan, fucoidan, laminarin, and extracts of Gracilaria tenuistipitata (GTE) and Sargassum duplicatum (SDE) examined the binding ability of LGBP with LPS and βG, as well as an increase of PO activity in shrimp haemocytes receiving a mixture of LGBP with LPS (rLVGBP-LPS) and a mixture of LGBP with βG (rLVGBP-βG), were concurrently conducted and served as reference groups.

2. Materials and methods

2.1. Experimental animals

White shrimp L. vannamei with an average weight of ~13 g obtained from the University Marine Station, Keelung, Taiwan were placed in aerated seawater in the laboratory for two weeks prior to the experiment. Only shrimp in the intermoult stage were used for the study. The moult stage was determined by examining the uropoda in which partial retraction of the epidermis could be distinguished (Chan et al., 1988).

2.2. Reagents

Alginate (A2158) derived from Macrocystis pyrifera, βG (G5011) derived from S. cerevisiae, carrageenan (C3889) derived from Gigartina aciculare, fucoidan (F5631) derived from Fucus vesiculosus, laminarin (L9634) derived from Laminaria digitata, LPS (L4005) derived from Escherichia coli (E. coli) O55:B5, and PG (69554) derived from Bacillus subtilis were obtained from Sigma Chemical Co., St. Louis, MO. Gracilaria tenuistipitata extract (GTE) and Sargassum duplicatum extract (SDE) were obtained following procedures described previously (Hou and Chen, 2005; Yeh et al., 2006). All of them are considered PAMPs and are used individually for binding and PO activity assays.

2.3. Construction and expression of recombinant rLVGBP protein

The cDNA fragment Lvlgbp was designed based on the full sequence of white shrimp L. vannamei lgbp (EU102286, Cheng et al., 2005b). The cDNA fragment of lgbp encoding the mature peptide of LGBP was amplified using Phusion High-Fidelity DNA polymerase (Thermo) with the specific primers rLVGBP F (5'-CCA TGG AAA TGA AGG GTG TCG TCG TGG CAT CGG TCT TGC TTC TG-3') and rLVGBP R (5'-CTC GAG CTG TTC GCC GCT GCT CAT CTA CCA GACG-3') containing Ncol and Xhol sites at their 5'-end, respectively. Purified PCR products were digested with Ncol and Xhol, ligated into the Ncol/Xhol sites of pET28b (+) expression vector (Novagen), and transformed into competent cells of E. coli JM109. Positive clones were confirmed by Sanger sequencing. The selected recombinant plasmid (pET28b-Lvlgbp) was transferred into E. coli BL21 (DE3) cells (Novagen) for recombinant protein expression.

The recombinant plasmid was then induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). After 3.5 h of induction, bacteria cells were centrifuged at 4000 g for 15 min. Precipitated cells were harvested, re-suspended with 1 mg ml⁻¹ lysozyme in a binding buffer (50 mM NaH₂PO₄, 300 mM NaCl, and 10 mM
Imidazole, pH 8.0), and disrupted with an ultrasonic oscillator (Sonicator XL2020, Misonix Incorporated). The suspension was then centrifuged, and its supernatant and precipitated matter evaluated for the presence of protein. After protein fraction analysis, rLvLGBP was found in inclusion bodies (precipitated matter). Precipitated matter was dissolved in urea to make a solution, centrifuged, and the supernatant passed through a column containing Nickel NTA Agar resin (ABT, Spain). The column was then washed several times with washing buffer (50 mM NaH2PO4, 300 mM NaCl, 20 mM Imidazole, and 8 M urea, pH 8.0) and rLvLGBP was then purified with elution buffer (50 mM NaH2PO4, 300 mM NaCl, 250 mM Imidazole, and 8 M urea, pH 8.0).

The resulting rLvLGBP protein was evaluated for purity through a SDS-polyacrylamide gel (SDS-PAGE). The protein was refolded using a refolding buffer from 6 M urea to 0 M urea for 24 h at 4°C following Laemmli (1970) and visualized with Coomassie brilliant blue R250. Supernatants were examined by SDS-PAGE and the concentration of rLvLGBP protein was determined based on Bradford’s method (Bradford, 1976).

For Western blot analysis, the rLvLGBP protein sample was resolved with a SDS-PAGE as above and then electro-blotted onto a PVDF membrane (American Biosciences). The membrane was blocked by incubation in blocking buffer (0.1% skim milk, 0.2% Tween 20, and 3 mM NaN3) and then probed with a 1:10,000 dilution of rabbit anti-rLvLGBP polyclonal antibody (GenScript) in blocking buffer, washed four times in TBST, and then probed with a 1:10,000 dilution of alkaline phosphatase-conjugated goat anti-rabbit IgG (Protech Technology Enterprise) secondary antibody in blocking buffer. The alkaline phosphatase antibody complex was detected by incubation in bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) Ready using Tablets (Roche) as the chromogenic substrate.

2.4. Binding assay of rLvLGBP with different PAMPs

The PAMPs binding assay was conducted following previously described procedures (Yu et al., 2006; Amparyup et al., 2012) with some modification. Briefly, 20 μg of LPS, βG, alginate, carrageenan, fucoidan, laminarin, GTE, and SDE in 100 μl TBS were used to coat each well of a 96-well microtiter plate (Costar) and air-dried overnight at 37°C. The plate was incubated at 60°C for 1 h to fix the ligands, and the wells were then blocked with 200 μl of 1 mg ml⁻¹ BSA in TBS at 37°C for 2 h. After washing, 100 μl of rLvLGBP (0–1 μM in TBS) was added to each well and incubated for 3 h at 28°C. After washing, the bound protein was detected immunochromatically. Briefly, 100 μl of a 1:5000 dilution of the rabbit anti-LGBP polyclonal antibody in TBS was added and incubated at 37°C for 3 h, washed, and then incubated for 3 h with 100 μl of alkaline phosphatase-conjugated-goat anti-rabbit IgG (diluted 5000-fold in TBS) as the secondary antibody. After each stage above the wells were washed four times with TBST (TBS with 0.1% (v/v) Tween 20) for 10 min and once with 0.5 mM MgCl2/10 mM diethanolamine. After the last wash, 200 μl of p-nitrophenyl phosphate (1.0 mg ml⁻¹ in the diethanolamine buffer) was added to each well and incubated at room temperature for 30 min. The reaction was stopped by adding 3 M NaOH (50 μl) and absorbance at 405 nm was measured with an ELISA reader (Bio-Tek, Winoski, VT, USA). Wells with 0 μg rLvLGBP protein (100 μl of TBS) were used as the negative control (blank). An apparent dissociation constant (Kd) values were calculated using Prism 5.00 software (GraphPad software) with a one-site binding model and non-linear regression analysis, as A = Amax [L]/(Kd + [L]), where A is the absorbance at 405 nm and [L] is the concentration of the rLvLGBP protein. In addition, in order to clarify whether free ligands of these PAMPs competitively inhibit the interaction between immobilized ligands and rLvLGBP, we conducted one more experiment. We examined whether free ligand of LPS competitively inhibits the interaction between immobilized βG and rLvLGBP. Two sets of experiments were conducted. The plate that coated with βG served as immobilized ligand, and the plate that coated with rLvLGBP, then LPS was added prior to first antibody reaction served as free ligand.

2.5. PO activity assay of rLvLGBP with different PAMPs

The PO activity of rLvLGBP in the white shrimp L. vannamei proPO system was determined by examining the formation of dopachromes produced from L-DOPA based on previously described methods (Hernández-López et al., 1996; Huynh et al., 2011; Chen et al., 2014). Six shrimp were used in the PO activity assay. There were 16 stimulant solutions (0.1 mg ml⁻¹ LPS, βG, alginate, carrageenan, fucoidan, laminarin, GTE, and SDE with or without rLvLGBP), one positive control (trypsin) and one negative control (CAC, background control). Each was separately dissolved in cacoodylate buffer (CAC). Briefly, haemolymph was individually withdrawn from the ventral sinus of shrimp, diluted with anticoagulant solution and placed in tubes for the PO activation assay following previously described procedures (Chen et al., 2014). Diluted hemolymph was added to the solution with or without the rLvLGBP protein (4 μM) and incubated at room temperature for 30 min. L-DOPA (3 mg ml⁻¹) was then added to each reaction and incubated at room temperature for 5 min. Shrimp PO activity at an optical density of 490 nm was measured with a spectrophotometer.

2.6. Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA). If significant differences were indicated at the 0.05 level, then Duncan’s multiple-comparison test was conducted to examine the significant differences among treatments using SAS computer software (SAS Institute, Cary, NC, USA). Statistically significant differences required that p < 0.05.

3. Results

3.1. Expression and enrichment of rLvLGBP

The recombinant plasmid pET28b-Lvlgbp was transferred to and expressed in E. coli BL21 (DE3). After IPTG induction for 3.5 h, whole cell lysate was analyzed by SDS-PAGE visualized by Coomassie Brilliant Blue R250 staining (data not shown). The rLvLGBP mature protein, after enrichment and refolding, appeared as a single band with an estimated molecular mass of ~42 kDa after SDS-PAGE resolution of the purified recombinant LvLGBP (Fig. 1A). Western blotting with the rabbit anti-LGBP polyclonal antibody revealed a single band of the same apparent size (Fig. 1B). Bovine serum albumin analysis indicated that the concentration of the rLvLGBP was 1282 μg ml⁻¹.

3.2. Binding of LvLGBP to LPS, βG, alginate, carrageenan, fucoidan, laminarin, GTE, and STE

We examined the binding activity of rLvLGBP with LPS, βG, alginate, carrageenan, fucoidan, laminarin, GTE, and SDE individually using ELISA. The rLvLGBP was bound to LPS and βG directly in a concentration-dependent manner in a saturable process (Figs. 2–4). The dissociation constants (Kd) of rLvLGBP bound to LPS and βG, calculated from saturation curve fitting according to the one-site binding model, were 0.1278 μM and 0.1216 μM, respectively (Fig. 2). The apparent dissociation constants (Kd) of rLvLGBP to alginate, carrageenan, fucoidan, laminarin, GTE, and SDE,
3. Phenoloxidase (PO) activity of rLvLGBP exposed to different PAMPS in vitro

We examined the PO activity of shrimp haemocytes incubated with the individual PAMP, rLvLGBP, and a mixture of rLvLGBP with each PAMP. No significant differences in PO activity were observed among the shrimp haemocytes incubated in rLvLGBP, LPS, βG, alginate, carrageenan, fucoidan, laminarin, STE, and SDE, and controls (CAC). However, the PO activity of shrimp haemocytes incubated in complexes of rLvLGBP and each PAMP were significantly higher than in shrimp haemocytes incubated with rLvLGBP, CAC or each PAMP alone. In the presence of LPS, βG, alginate, carrageenan, fucoidan, laminarin, STE, and SDE, rLvLGBP significantly enhanced PO activity by 328%, 172%, 200%, 213%, 197%, 194%, 191%, and 197%, respectively (Figs. 6–8).

4. Discussion

The full-length sequence of white shrimp LGBP cDNA was characterized with an open reading frame of 1101 bp, encoding 367 amino acids (aa) that contains a signal peptide of 17 aa residues. The calculated molecular mass of the 350 aa mature peptide was 41.56 kDa whereas that of rLvLGBP in the present study was ~42 kDa.

Scientists have studied the LGBP transcript level of shrimp receiving *Vibrio* or LPS. For instance, the LGBP transcript level of white shrimp *L. vannamei* is increased at 3 h after a *Vibrio alginolyticus* challenge (Cheng et al., 2005b). The LGBP transcript level of tiger shrimp *P. monodon* is increased at 24 h after a *V. harveyi* challenge (Amparyup et al., 2012). The LGBP transcript level of Indian white shrimp *Fenneropenaeus indicus* is increased at 3 h after a *Vibrio parahaemolyticus* challenge (Valli and Vaseeharan, 2012). The LGBP transcript level of fleshy shrimp *F. chinensis* is increased at 6 h after challenging a mixture of *Vibrio anguillarum* and *Staphylococcus aureus* (Liu et al., 2009). The LGBP transcript level of kuruma shrimp *M. japonicus* is increased at 12–48 h after a LPS challenge (Lin et al., 2008). These facts indicate that the transcript level of LGBP is increased in response to *Vibrio* and LPS challenges after 3–24 h.

Scientists have studied the transcript levels of βGBP or LGBP of shrimp receiving βG or seaweed polysaccharide. For instance, the βGBP transcript level of tiger shrimp fed a diet containing sodium alginate at 1.0 g kg$^{-1}$ is increased after five months (Liu et al., 2006). The LGBP transcript level of white shrimp fed a diet containing βG at 2 g kg$^{-1}$ is increased after three days (Wang et al., 2008). The LGBP transcript level of white shrimp fed a diet containing carrageenan at 0.5 g kg$^{-1}$ is increased after three weeks (Chen et al., 2014). The LGBP transcript level of white shrimp receiving fucoidan at 10 μg g$^{-1}$ via injection is increased after 12 h (data not shown). The LGBP transcript level of white shrimp immersed in seawater containing GTE at 600 mg L$^{-1}$ is increased after 3 h (Chen et al., 2015). Therefore, shrimp receiving βG, alginate, carrageenan, fucoidan, and GTE all increased their transcript levels of LGBP or βGBP.

Degranulation is induced in crayfish and crab haemocytes when incubated with laminarin (Smith and Söderhäll, 1983; Smith et al., 1984). Degranulation is induced in white shrimp haemocytes incubated in fucoidan (Kitikiew et al., 2013). Crayfish haemocytes...
incubated with LPS or zymosan have caused reduced percentages of large cells and increased percentages of small cells (Cardenas et al., 2000, 2004; Xian et al., 2009). White shrimp haemocytes incubated with fucoidan and carrageenan have reduced percentages of large cells and increased percentages of small cells (Kitikiew et al., 2013; Chen et al., 2014).

In vitro PO activity increases in crayfish haemocytes incubated with LPS, curdlan, and laminarin (Lee et al., 2000). Shrimp haemocytes incubated with alginate, curdlan, fucoidan, carrageenan, laminarin, and Sargassum extract all show increases in PO activity and superoxide anion (Huynh et al., 2011; Kitikiew et al., 2013; Chen et al., 2014). Therefore, shrimp haemocytes receiving LPS, βG, alginate, curdlan, fucoidan, and laminarin exhibit haemocyte degranulation, a change in haemocyte morphology, and an increase in PO activity.

Scientists have studied the binding ability of βGBP and LGBP with βG. For instance, tiger shrimp P. monodon βGBP binds to curdlan, zymosan, but LPS could not bind to βGBP in immunoblotting (Sritunyalucksana et al., 2002). Crayfish P. leniusculus LGBP has binding activity to LPS, curdlan, and laminarin, but peptidoglycan (PG) could not bind to LGBP (Lee et al., 2000). The complex of βGBP and βG increases the activities of proPO activating enzyme (ppAE) and PO in crayfish P. leniusculus and brown shrimp Penaeus californiensis (Duvic and Söderhäll, 1990; Vargas-Albores et al., 1996). PO activity significantly increases in shrimp haemocytes incubated with a mixture of purified βGBP from Brazilian shrimp Farfantepeneaus paulensis and L. schmitti, plus laminarin, compared to shrimp haemocytes incubated with either βGBP or laminarin alone (Goncalves et al., 2012). Therefore, shrimp haemocytes receiving βG or laminarin bind with βGBP, subsequently triggering the proPO activating system.

Tiger shrimp P. monodon recombinant LGBP (rPmLGBP) binds to LPS and βG, and the PO activity of shrimp haemocytes incubated with a complex of rPmLGBP-LPS or a complex of rPmLGBP-βG is significantly higher than in shrimp incubated with rPmLGBP, LPS, or βG alone (Amparyup et al., 2012). In tiger shrimp P. monodon,
The complex of rβ-glucan and rLPS exhibited much higher PO activity compared to shrimp haemocytes incubated with rβ-glucan or rLPS alone. This suggests that the complex of rβ-glucan and rLPS may bind to rLGBP and alginate. (B) Phenoloxidase (PO) activity of white shrimp Litopenaeus vannamei haemocytes incubated with laminarin and rLGBP-GTE. (C) Phenoloxidase (PO) activity of white shrimp L. vannamei haemocytes incubated with fucoidan and a complex of rβ-glucan and rLGBP-LPS, and rLGBP-βG was much higher than in shrimp haemocytes incubated with rLGBP and each polysaccharide only. Therefore, shrimp haemocytes incubated with a mixture of rLGBP and PAMP showed much higher PO activity compared to shrimp haemocytes incubated with PAMP only. These facts support the recognition and binding of LGBP with PAMPs (including alginate, carrageenan, fucoidan, laminarin, and SDE) that subsequently triggers the proPO system and indicates that the activation of innate immunity has occurred.

Similarly to the binding ability of rβ-glucan to βG, we found that rLGBP is able to recognize and bind LPS and βG in the present study. Furthermore, the Kd of rLGBP bound to βG in the presence of free ligand of LPS was higher than that of rLGBP bound to βG. This fact suggests that LPS affects affinity of rLGBP binding to βG. This fact suggests that the complex of rLGBP-LPS may prevent the binding of rLGBP with βG, and also suggests that βG and LPS may bind to rLGBP at the same domain.
βG, curdlan, and zymoan are β-1,3-glucosidic linked polymer (Sritunyaualucksana et al., 2002). LPS is a complex of lipid and polysaccharide that is mainly composed of mannose, rhamnose, galactose, and glucose. Alginate is a polymer of β-(1,4)-α-mannuronic acid and α-(1,4)-γ-guluronic acid, carrageenan is a polymer of α-(1,3)-galactose acid and β-(1,3,6)-anhydro-α-galactose, fucoidan is a polymer of (1,2)-α-L-fucose-4-sulphate, and laminarin is a polymer of (1,3)-β-D-glucosyl with (1,6)-β-D-glucose (Jimenez-Escrig and Sanchez-Muniz, 2000). The main polysaccharide components of GTE are galactose, fucose, fructose and glucose, and the main polysaccharide component of STE are fucose, galactose, and nicotinic acid and a polymer of (1,3)-β-D-glucan. Escrig and Smatory, 2000). The main polysaccharide component of STE are fucose, galactose, and the main polysaccharide component of STE are fucose, galactose, and fructose (Chen et al., 2015). Early studies indicated that when crayfish IGBP binds to βG, it becomes activated and binds specifically to a cell-surface associated protein, a superoxide dismutase, or binds to a β-integrin on the haemocyte surface through RGD motif (Holmblad and Söderhäll, 1999; Johansson et al., 1999; Jiravanichpaisal et al., 2006). RGD and KGD, common integrin-binding motifs are observed in LGBP and peroxinectin in white shrimp L. vannamei (Li et al., 2004; Cheng et al., 2005b). Integrin β plays important roles in propO activation, phagocytosis and antioxidant system (Lin et al., 2013). The transcript levels of LGBP, peroxinectin, integrin β, and mtMnSOD are up-regulated in haemocytes of white shrimp that received 600 mg l⁻¹ GTE after 3 h (Chen et al., 2015). The transcript levels of LGBP, peroxinectin, integrin β, prophenoloxidase, cytMnSOD and mtMnSOD are up-regulated in haemocytes of white shrimp that fed a diet containing carrageenan at 0.5 g kg⁻¹ after 3 weeks (Chen et al., 2014). Peroxinectin has cell adhesion and is associated with integrin β in mediating the binding of haemocytes, PAMP and PRP (Cerenius et al., 2008). Further research is needed to clarify binding of rLGBP with each polysaccharide becomes activated, and binds cell-surface protein and integrin β. In addition to LGBP and IGBP, a C-type lectin has been identified from white shrimp L. vannamei, and the recombinant protein exhibits agglutination with E. coli (Zhang et al., 2009). A ML superfamily protein has been identified from white shrimp, and enzyme-linked immunosorbent assays show that recombinant LvML binds to LPS (Liao et al., 2011). However, we do not know whether C-type lectin, ML protein, or other PRPs in white shrimp show recognition and binding to LPS, βG, or seaweed polysaccharide.

In conclusion, LGBP is an important PRP in white shrimp L. vannamei. Recombinant LGBP (rLGBP) binds to alginate, carrageenan, fucoidan, laminarin, GTE, and STE as well as LPS and βG, and leads to an increase in PO activity. Shrimp haemoocytes receiving seaweed polysaccharides and seaweed extract induce the recognition and binding of LGBP, cause degranulation, and activate the proPO system that subsequently leads to an increase in PO activity, indicating the activation of innate immunity.

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Fig. 8. (A) Phenoloxidase (PO) activity of white shrimp Litopenaeus vannamei haemoocytes incubated with Gracilaria tenuistipitata extract (GTE) and a complex of rLGBP and GTE. (B) Phenoloxidase (PO) activity of white shrimp L. vannamei haemoocytes incubated with Sargassum duplicatum extract (SDE) and a complex of rLGBP and SDE.
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