Characterization of prostanoid pathway and the control of its activity by the eyestalk optic ganglion in the female giant freshwater prawn, *Macrobrachium rosenbergii*

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**ABSTRACT**

The giant freshwater prawn, *Macrobrachium rosenbergii*, is an economically valuable species that are distributed throughout the Asia-Pacific region. With the natural population declining due to overfishing, aquaculture of this species is deemed necessary. Hence, it is essential to understand the mechanisms regulating reproduction in order to increase their production. Prostaglandins (PGs) play an important role in reproduction in most vertebrates and several invertebrates. It has been proposed that crustaceans have PGs but the prostanoids pathway in the giant freshwater prawn is still unclear. In this study, we identified 25 prostanoid-related genes involved in the biosynthesis of active prostanoids in *M. rosenbergii* using in silico searches of transcriptome data. Comparative analysis of encoded proteins for the *MrOPEG2* gene with other species was performed to confirm their evolutionary conservation. Gene expression analysis revealed the correlation of *MrOPEG2* gene expression level with the progress of ovarian development. Eyestalk ablation increased the expression level of *MrOPEG2* gene compared to intact groups during the ovary maturation stages. Collectively, this study confirmed the existence of prostanoids in the giant freshwater prawn, as well as characterizing key gene *MrOPEG2* associated with the prostanoid pathway. We propose that *MrOPEG2* may play an important role in *M. rosenbergii* ovarian maturation and its expression is under the inhibitory control from the eyestalk optic ganglion hormones. Identification of genes in prostanoid pathway and their expressions enables future functional studies to be performed, which may lead to applications in the aquaculture of this species.

1. Introduction

*Macrobrachium rosenbergii*, the giant freshwater prawn, has been highly valued as food in the tropical countries of Asia for domestic consumption and export (Hossain and Das, 2010; New and Nair, 2012). The demand for this prawn is rapidly increasing and necessitates the production of this species by aquaculture. Understanding the processes that control gonadal maturation and gamete production are key to success in aquaculture of this species. Reproduction of the giant freshwater prawn as well as other crustaceans is a highly complex process that is controlled by neuroendocrine factors (Nagaraju, 2011; Subramoniam, 2011). Manipulation actors of some key factors may help to increase their reproduction by aquaculture (Okumura, 2004).

Prostaglandins (PGs) play important roles in several essential physiological processes including reproduction in most animals (Rowley et al., 2005; Wimuttisuk et al., 2013). The biosynthesis of prostanoids from their precursors occurs through the action of key enzymes, especially cyclooxygenase (COX) and specific terminal prostanoids synthases.
2.2. Animals and tissue collection

All methods were carried out in accordance with relevant guidelines and regulations for using animals. All the experimental procedures presented in this work were approved by the Animal Care and Use Committee of Walailak University, National Research Council of Thailand (NRCT), Protocol No. 005/2019.

Live mature female M. rosenbergii were obtained from a Phran Nok market, Bangkok, Thailand. They were then acclimatized in culture tanks at the Faculty of Science, Mahidol University. These included mature female prawns with different stages of the ovarian cycle [stages 1–4, as described previously (Meeratana and Sobhon, 2007); 20 prawns/stage; n = 80, with average weight of 30–40 g, and mature male prawns, n = 20, with average weight of 150–200 g. After 24 h in culture tanks, the animals were anesthetized by immersion in ice-cold water for 5 min before sacrifice. The eyestalks, brains, thoracic ganglia, abdominal ganglia, ovaries, testes, hepatopancreases, hematopoietic tissues, guts, hearts, and muscles were collected and immediately frozen in liquid nitrogen, then stored at −80 °C until preparation of total RNA.

2.3. Total RNA extraction

Frozen tissues were individually homogenized and total RNA extracted with TRIzol reagent (Thermo Fisher Scientific, MA, USA) following the manufacturer’s protocol in combination with a DNaseI (Thermo Fisher Scientific, MA, USA) treatment to eliminate potential genomic DNA contamination. The quantity and quality of RNA samples were measured using spectrophotometry (NanoDrop 1000; Thermo Fisher Scientific, DE, USA). Total RNA of each tissue was pooled and dried separately.

2.4. Tissue expression of MroPGES2 by RT-PCR

Two micrograms of total RNA of each tissue were used for cDNA synthesis. Complementary DNA (cDNA) was generated by reverse transcription of total RNA using RevertAid RT kit (Thermo Scientific, USA) following the manufacturer’s protocol. Gene-specific primers for MroPGES2 gene were designed using the Primer-BLAST program (https://www.ncbi.nlm.nih.gov/tools/primer-blast) (Ye et al., 2012) (Table 1). PCR was carried out using the PCR SuperMix (Thermo Fisher Scientific, MA, USA) following a routine protocol optimized for the primers. Beta-actin gene was used as a positive control, while the negative control was non-RT cDNA. PCR products were analyzed by agarose gel electrophoresis. The amplicons of expected size were extracted by QIAquick gel extraction kit (Qiagen, Hilden, Germany) and subcloned into the pDrive vector (Qiagen, Hilden, Germany). The plasmids were purified by QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany), and sequenced by Macrogen (Macrogen Ltd., Seoul, South Korea). The obtained sequences were analyzed using multiple bioinformatics tools including BLAST against the NCBI GenBank database, and the putative amino acid sequence was deduced by using Expasy bioinformatics tool (http://web.expasy.org/translate/) (Artimo et al., 2012).

Table 1. Gene-specific primers used for tissue expression and quantitative real-time PCR.

| Genes          | Techniques | Forward primer (5’-3’) | Reverse primer (5’-3’) | Size (bp) |
|----------------|------------|------------------------|------------------------|-----------|
| MroPGES2       | RT-PCR     | TGACTCGGGCACAAACCAAGA  | TGGAGTCTCTGAAAACATCAC  | 560       |
|                | qPCR       | AGATGAAAGGAGATGGGACA   | GCTGCGGACACATACAAACA  | 180       |
| Beta-actin     | RT-PCR/qPCR| GCAGGAGATGACACGCAGAA  | GATGCGGACAGATTCATA    | 152       |
| Prostanoid-related genes                  | Transcript                        | ES (TPM value) | CNS (TPM value) | Ov (TPM value) | BLAST hit and species                        | E-value   | Accession numbers |
|------------------------------------------|------------------------------------|----------------|----------------|---------------|-----------------------------------------------|-----------|------------------|
| Cytosolic phospholipase A2 (cPLA2)       | Unigene19244_All, Unigene7080_All  | 5.95           | 14.72          | 0.38          | Cytosolic phospholipase A2 [Penaeus monodon]   | 0         | AFJ11391.1       |
| Secreted phospholipase A2 (sPLA2)       | Unigene6650_All                    | 10.69          | 36.54          | 1.98          | Group 3 secretory phospholipase A2 [Stegodyphus mimosarum] | 4E-42     | KFM61512.1       |
| Phospholipase C delta (PLCd)            | Unigene7737_All                    | 6.99           | 15.59          | 1.86          | Phospholipase C delta, putative [Erodex scapularis] | 2E-94     | XP_002410916.1   |
| Phospholipase C beta (PLCb) isoform 1    | Contig19390_McCNS                 | 4.68           | 13.09          | 0.91          | Phospholipase C beta 1 [Bombyx mori]          | 4E-165    | AAD02690.1       |
| Phospholipase C beta (PLCb) isoform 2    | Unigene36025_All                   | 12.24          | 1.17           | 0.34          | Phospholipid phospholipase C beta isoform [Homarus americanus] | 0         | AAD02690.1       |
| Phospholipase C gamma (PLCg)            | Unigene57328_All                   | 0.34           | 0.67           | 8.65          | Phospholipase C gamma [Bombyx mori]           | 3E-119    | NP_001165394.1   |
| Cyclooxygenase 1 (COX1)                 | Unigene20053_All, Unigene27866_All| 12.98          | 15.21          | 0.69          | Cyclooxygenase [Halocaridina rubra]           | 0         | ALG96666.1       |
| Cyclooxygenase 2 (COX2)                 | Unigene22278_All                   | 6.10           | 13.39          | 24.22         | Cyclooxygenase [Halocaridina rubra]           | 0         | ALG96666.1       |
| Glutathione-dependent prostaglandin D synthase (gPGDS) isoform 1 | Unigene3543_All                   | 10.62          | 12.97          | 3.86          | Glutathione-dependent prostaglandin D synthase [Penaeus monodon] | 5E-95     | AFJ11393.1       |
| Glutathione-dependent prostaglandin D synthase (gPGDS) isoform 2 | Unigene3451_All                   | 21.71          | 56.72          | 5.44          | Glutathione-dependent prostaglandin D synthase [Penaeus monodon] | 3E-27     | AFJ11393.1       |
| Hematopoietic prostaglandin D synthase (hPGDS) isoform 1 | Unigene20033_All                   | 27.70          | 374.43         | 55.93         | Hematopoietic prostaglandin D synthase [Penaeus monodon] | 3E-74     | AFJ11392.1       |
| Hematopoietic prostaglandin D synthase (hPGDS) isoform 2 | Unigene19478_All, Unigene19813_All| 9.08           | 5.93           | 31.86         | Hematopoietic prostaglandin D synthase [Penaeus monodon] | 2E-52     | AFJ11392.1       |
| Hematopoietic prostaglandin D synthase (hPGDS) isoform 3 | Unigene19813_All                   | 3.29           | 0.43           | 4.15          | Hematopoietic prostaglandin D synthase [Penaeus monodon] | 9E-22     | AFJ11392.1       |
| Prostaglandin E synthase (cPGES)        | Unigene7084_All                    | 35.98          | 67.13          | 126.43        | Cytosolic prostaglandin E synthase [Penaeus monodon] | 2E-67     | AFJ11394.1       |
| Microsomal prostaglandin E synthase (mPGES) isoform 1 | Unigene17352_All                   | 9.06           | 33.92          | 4.91          | Microsomal prostaglandin E synthase [Penaeus monodon] | 1E-57     | AFJ11395.1       |
| Microsomal prostaglandin E synthase (mPGES) isoform 2 | Unigene49139_All                   | 1.85           | 1.45           | 4.56          | Microsomal prostaglandin E synthase [Penaeus monodon] | 1E-29     | AFJ11395.1       |
| Prostaglandin E synthase 2 (PGES2)      | Unigene3911_All, Unigene19799_All  | 5.13           | 5.88           | 71.86         | Prostaglandin E synthase 2 [Penaeus monodon]   | 0         | AFJ11396.1       |
| Prostaglandin F synthase 1 (PGFS1)      | Unigene26298_All                   | 25.35          | 38.56          | 299.44        | Prostaglandin F synthase [Penaeus monodon]     | 0         | AFJ11397.2       |
| Prostaglandin F synthase 2 (PGFS2)      | Unigene23531_All                   | 1.80           | 2.97           | 14.37         | Prostaglandin F synthase-like [Aplysia californica] | 2E-103    | XP_005089972.1   |
| Thromboxane A synthase isoform 1        | Unigene48062_All                   | 1.05           | 1.94           | 113.63        | Thromboxane-A synthase-like [Oreochromis niloticus] | 1E-84     | XP_013127958.1   |
| Thromboxane A synthase isoform 2        | Unigene45852_All                   | 0.83           | 4.37           | 0.34          | Thromboxane A synthase-like protein [Daphnia pulex] | 1E-52     | EFX87565.1       |
| Thromboxane A synthase isoform 3        | Unigene46960_All                   | 0.00           | 2.96           | 0.53          | Thromboxane A synthase [Penaeus monodon]        | 6E-21     | AFJ11398.1       |
| 15-hydroxyprostaglandinidehydrogenase   | Contig5577_McCNS                   | 1.89           | 6.91           | 0.36          | 15-hydroxyprostaglandinidehydrogenase-like [Lingula anatina] | 2E-64     | XP_013396931.1   |
| Aldo-keto reductase                     | Unigene299_All                     | 5.74           | 7.44           | 37.30         | Aldo-keto reductase 1 [Coptotermes gestroi]     | 4E-113    | AM219949.1       |
| Carbonyl reductase                      | Unigene10725_All                   | 13.45          | 23.40          | 68.45         | Carbonyl reductase 1 [Zootermopsis nevadensis]   | 2E-121    | KDR03826.1       |
stored at -80 
the entire ovarian cycle, immediately frozen in liquid nitrogen, and eyestalk ablated female prawns in each stage were collected throughout cautery and antibiotic pomades. The ovarian tissues of intact and to the base with a scalpel. The incision spot was closed with an electric ment. Each ablated female prawn had one of its eyestalks removed close acclimatized in a culture tank for 2 
were separated into 2 groups; intact and eyestalk ablated. They were determinations of the expression of MroPGES2 in each ovarian stage were performed using a DXM1200F digital microscope (Nikon, Tokyo, Japan). were observed and photographed under Nikon E600 microscope equip- ous described protocol (Thongbuakaew et al., 2019). The stained sections was detected by ISH. Brie-ly, the ovaries were dissected out and fixed in fresh 4% paraformaldehyde fixative in 0.1M PBS, pH 7.4 at 4 °C overnight. Then the tissues were processed by routine paraffin method. Paraffin embedded blocks were sectioned at 6 μm thickness. MroPGES2 gene was PCR-amplified with M13 primers using the plasmid containing the MroPGES2 gene as a template (forward: 5’ GTAAACGACGCGCCAGT 3’ and reverse primer, 5’ AACACGTATGACCATG 3’). The PCR products were processed through separation and extraction using QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) and used as a template for riboprobe synthesis using a DIG-oligonucleotide labeling kit (Roche, Germany). The in situ hybridization was performed following the previous described protocol (Thongbuakaew et al., 2019). The stained sections were observed and photographed under Nikon E600 microscope equipped with a DXM1200F digital microscope (Nikon, Tokyo, Japan).

2.5. Expression of MroPGES2 in developing oocytes by in situ hybridization (ISH)

The spatial distribution of PGESs expression in the ovarian tissue sections was detected by ISH. Briefly, the ovaries were dissected out and fixed in fresh 4% paraformaldehyde fixative in 0.1M PBS, pH 7.4 at 4 °C overnight. Then the tissues were processed by routine paraffin method. Paraffin embedded blocks were sectioned at 6 μm thickness. MroPGES2 gene was PCR-amplified with M13 primers using the plasmid containing the MroPGES2 gene as a template (forward: 5’ GTAAACGACGCGCCAGT 3’ and reverse primer, 5’ AACACGTATGACCATG 3’). The PCR products were processed through separation and extraction using QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) and used as a template for riboprobe synthesis using a DIG-oligonucleotide labeling kit (Roche, Germany). The in situ hybridization was performed following the previous described protocol (Thongbuakaew et al., 2019). The stained sections were observed and photographed under Nikon E600 microscope equipped with a DXM1200F digital microscope (Nikon, Tokyo, Japan).

2.6. Determinations of the expression of MroPGES2 in each ovarian stage of intact and eyestalk ablated female prawns by real-time PCR

2.7. Statistical analysis

3. Results

3.1. Gene mining and identification of prostanoid-related genes

We used a de novo assembled transcriptome for M. rosenbergii to identify prostanoid-related genes. We found 25 transcripts encoding prostanoid-related genes, including members of the enzymes involved in prostanoid pathway. Sequence and annotation information is provided in Table 2. Moreover, Figure 1 illustrates the biosynthesis pathway position of the enzyme genes identified that are involved in the biosynthesis of active prostanoids in M. rosenbergii.

3.2. Characterization of MroPGES2

MroPGES2 transcript encoding a full-length protein is composed of 413 aa (Figure 2A). MroPGES2 contains conserved membrane-anchored dimeric domain (Cys-x-x-Cys) and GSH-binding domain (Figure 2A-B). At C-terminal, MroPGES2 also contains glutathione S-transferase domain (Figure 2A-B). Moreover, MroPGES2 contains 4 conserved cysteine residues predicted to form 2 disulfide bridges (Figure 2A-B). Alignment transcription was performed on the Applied Biosystems 7500 Fast Real-Time PCR (Applied Biosystems, CA, USA) following a protocol optimized for MroPGES2 gene with dissociation curve analysis. Transcripts were quantified using a standard curve method (Larionov et al., 2005).
between MroPGES2 with other species homologs demonstrates conservation within the key motifs (Figure 3A). The MroPGES2 clusters with homologs of crustaceans and insects and is clearly distinguished from vertebrate homologs (Figure 3B).

3.3. Tissue expression of MroPGES2 and spatial distribution of MroPGES2 in developing oocytes

MroPGES2 expression was observed in all investigated tissues including, eyestalk, brain, thoracic ganglion, abdominal ganglion, hematopoietic tissue, hepatopancreas, ovary, muscle, heart, gut and testis but more abundantly expressed in ovary. However, signal was weak level in eyestalk and hepatopancreas (Figure 4).

Next, we determined expression and spatial distribution of MroPGES2 in developing oocytes by ISH, and found positive signal in the cytoplasm of oogonia (Og), previtellogenic (including Oc1 and Oc2) and early vitellogenic (Oc3) oocytes (Figure 5A1-A3), and follicular cell type 1 (Fc1) and 2 (Fc2) (Figure 5B1-B3). Strong positive staining was observed in previtellogenic oocytes, whereas the Og and Oc3 showed less intense staining (Figure 5A3). No gene expression was observed within the late vitellogenic oocyte (Oc4) and mature oocytes (mOc). As well no signal was detected in the negative control in which MroPGES2 sense riboprobes were used (Figure 5C1-C3).

3.4. Expression of MroPGES2 in each ovarian stage of intact and eyestalk ablated female prawns

We further analyzed the relative gene expression in the ovarian tissues of intact and eyestalk ablated female prawns during gonad maturation by real-time PCR (Figure 6). Results revealed that the expression of oogonia (Og), previtellogenic (including Oc1 and Oc2) and early vitellogenic (Oc3) oocytes (Figure 5A1-A3), and follicular cell type 1 (Fc1) and 2 (Fc2) (Figure 5B1-B3). Strong positive staining was observed in previtellogenic oocytes, whereas the Og and Oc3 showed less intense staining (Figure 5A3). No gene expression was observed within the late vitellogenic oocyte (Oc4) and mature oocytes (mOc). As well no signal was detected in the negative control in which MroPGES2 sense riboprobes were used (Figure 5C1-C3).
*MroPGES2* gradually increased from stage 1 to stage 3 of ovarian development and decreased at mature stage (stage 4). *MroPGES2* gene expression was markedly increased in the stage 3 of ovarian development. Importantly, eyestalk ablated female prawns showed higher expression level of *MroPGES2* at all stages, and markedly at stages 2 and 3, of the ovarian cycle when compared to intact female prawns.

**4. Discussion**

Our present study has proven the existence of prostanoid pathway in the giant freshwater prawn, *M. rosenbergii*, as well as genes involved in the biosynthesis of active prostanoids. The occurrences of PGs biosynthesis in invertebrate animals have been established in insects (Stanley,
2006; Stanley and Kim, 2014) and aquatic invertebrates (Rowley et al., 2005). Fully constructed eicosanoid biosynthesis pathway in the Daphnia has been reported based on bioinformatic and transcriptomic evidences, which revealed nine prostanoid biosynthetic genes (Heckmann et al., 2008). Moreover, COX and PGES have been identified in amphipod crustaceans, Gammarus spp. and Caprella spp. (Varvas et al., 2009; Hansen et al., 2014). The putative prostanoid pathway has been shown in the black tiger shrimp, P. monodon, which contains only nine prostanoid biosynthesis genes (Wimuttisuk et al., 2013). Our study demonstrated that M. rosenbergii has the same types of prostaglandin synthase enzymes and prostanoid pathway as those found in vertebrates and other species.

PGE2 is the most common prostanoid, which is converted from PGH2 by PGES enzyme, and it plays an important role in a variety of actions, including reproductive functions in vertebrates (Sun et al., 2006; Milatovic et al., 2011) and invertebrates (Stanley, 2006; Stanley and Kim, 2014; Sumpownik et al., 2015; Duangprom et al., 2018). In the present study, the sequence of MroPGES2 in the M. rosenbergii was identified and its deduced amino acid sequence was predicted. Sequence comparison by amino acid alignment of PGES2 proteins from several species indicated that PGES2 are relatively conserved, especially at the key motifs, which comprised of the membrane-anchored dimeric domain (CyxxCyx) and GSH-binding motif. Based on a human PGES2 model (Sjögren et al., 2013), silkworm Bombyx mori (Yamamoto et al., 2013), the penaeid shrimp, P. monodon (Wimuttisuk et al., 2013), and lepidopteran insect, Spodoptera exigua (Ahmed et al., 2018), these domains are responsible for catalyzing the isomerization of PGH2 to PGE2. Phylogenetic comparison of the MroPGES2 precursors with other sequences revealed that MroPGES2 clusters with invertebrates and most closely related to other crustaceans. However, invertebrate PGESs forms distinct clades from vertebrates due to low heme-binding affinity when compared with vertebrate PGESs (Hansen et al., 2014). This suggested that MroPGES2 is responsible for PGE2 biosynthesis in M. rosenbergii and shares a common ancestor with vertebrates and other species.

By RT-PCR we found the expression of MroPGES2 mRNA in all organs studied but the expression was strongest in ovary. Investigation of spatial gene expression by ISH indicated that MroPGES2 was expressed in various developing oocytes and follicular cells but not in late vitellogenic oocyte (Oc4). This pattern of expression implied that in the M. rosenbergii, MroPGES2 might be involved in controlling early oocyte development and ovarian maturation. Similarly, PGES2 mRNA was localized in various tissues and in the oocytes and follicular cells of the mud crab, Scylla.

Figure 4. Agarose gel showing tissue-specific expression of Macrobrachium rosenbergii PGES2 gene using RT-PCR with specific primers. The beta-actin gene was used as a positive control. Negative control was performed without cDNA. The expected amplicon size is shown in base pairs (bp). Full images of gels are shown in S2.

Figure 5. In situ hybridization localization of MroPGES2 mRNA transcripts in the ovary of the Macrobrachium rosenbergii. (A1–A3) Localized expression of MroPGES2 in the cytoplasm of oogonia (Og), previtellogenic oocytes (Oc1 and Oc2) and vitellogenic oocytes (Oc3). (B1–B3) High magnification showing positive staining in the cytoplasm of Oc1, Oc2, Oc3, respectively, and follicular cell type 1 (green asterisks) and type 2 (yellow asterisks). (C1–C3) Negative control micrograph using a DIG-labeled sense-strand MroPGES2 riboprobes showing no positive signal in the ovary. Scale bars represent 250 μm (A1, C1), 100 μm (A2, C2), 25 μm (A3, C3), 10 μm (B1–B3). Abbreviations: Oogonia (Og); Oocyte 1 (Oc1); Oocyte 2 (Oc2); Oocyte 3 (Oc3); Oocyte 4 (Oc4).
olivacea, and that PGES may be involved with oocyte development in crab (Duangprom et al., 2018). As well, the presence of PGES2 in the ovary of the penaeid shrimp, P. monodon, suggested its possible involvement in the oocyte development (Preechaphol et al., 2010). In the freshwater crab, O. senex senex, PGE2 was detected in many tissues, including ovaries with greater gene expression during vitellogenesis (Reddy et al., 2004). Moreover, PGE2 was detected by immunoperoxidase at relatively higher level in the Oc1 and Oc2 than other stages of oocytes of the giant freshwater prawn, M. rosenbergii (Sumpownon et al., 2015), and in the early stage of oocytes of the kuruma prawn, M. japonicus by use immunoenzyme assay (Tahara and Yano, 2004). These findings suggested PGE2 plays specific role in oocyte development especially in vitellogenesis (Tahara and Yano, 2004; Sumpownon et al., 2015). Moreover, PGE2 also plays a role in the mediating oocyte maturation in the zebrafish (Lister and Van Der Kraak, 2008). Taken together, these findings suggest a possible and important role of PGES2 and PGE2 in controlling oocyte development. Their roles in other processes of female reproduction such as spawning in crustaceans has also been suggested (Tahara and Yano, 2004; Sumpownon et al., 2015).

By using qPCR, we found that the amount of MroPGES2 gene expression increased as the ovary developed and decreased when it reached mature stage. This is consistent with the previous study in the giant freshwater prawn, M. rosenbergii, which reported that PGE2 level in the ovaries was high during early stages of the ovarian cycle and subsided in late stages (Sumpownon et al., 2015). As well, the amount of PGES2 gradually increased during ovary development in the mud crab, S. olivacea (Duangprom et al., 2018). The concentration of PGE2 was strongly correlated with ovarian maturation in the crayfish, P. paeninsulanus (Spaziani et al., 1993, 1995), the freshwater edible crab, O. senex senex (Reddy et al., 2004), the kuruma prawns, P. japonicas (Tahara and Yano, 2004), and the penaeid shrimp, P. monodon (Preechaphol et al., 2010). Furthermore, in eyestalk-ablated prawns the expression level of MroPGES2 was increased in early stages of ovarian maturation when compared to intact prawns, and the increases were most notable during stages 2 and 3. As it was well known that the eyestalk optic ganglia secrete many important hormones involved in ovarian maturation, including GH which inhibits the synthesis of vitellogenin (Treeratrakool et al., 2008; Nagaraju, 2011; Uawisetwathana et al., 2011). Removing eyestalk induced early ovarian maturation in many decapods (Okumura et al., 2006; Okumura, 2007; Fernandez and Radhakrishnan, 2016) by removing GH as demonstrated in the pink shrimp, Peneaus notialis (Rosas et al., 1993). Moreover, eyestalk ablation significantly increased vitellogenin expression level in the hepatopancreas and ovary resulting in acceleration of ovary maturation by removing inhibitory hormone in the oriental river prawn, Macrobrachium nipponense (Bai et al., 2015). Known reproductive genes involved in ovarian maturation were dramatically increased after removing eyestalk in the black tiger shrimp, P. monodon (Uawisetwathana et al., 2011). Furthermore, RNA interference of GH reduced the GH transcript level resulting in stimulation of ovarian maturation in P. monodon (Treeratrakool et al., 2008). Thus, we suggested that MroPGES2 expression might be controlled by GH from the eyestalk.

5. Conclusion

In conclusion, a putative prostanoid pathway and prostanoid-related genes in the M. rosenbergii had been demonstrated, and they showed conservation with crustaceans and other species. MroPGES2 and PGE2 expressions in ovaries were high during early stages of the ovarian cycle as well as in early oocytes, but subsided when the ovaries reached maturity and the oocytes became fully developed. Thus, MroPGES2 and PGE2 might be involved in oocyte development and vitellogenesis. Eyestalk ablation shortened the period of ovarian maturation and significantly enhanced the levels of MroPGES2 expression in the ovaries compared to those of the unablated prawns. Thus, the inhibitory eyestalk hormones, possibly GH might control the expression of MroPGES2. This knowledge on prostanoid pathway and roles of MroPGES2 and PGE2 may be applied to increase the production of this species in aquaculture.

Declarations

Author contribution statement

T. Thongbuakaw: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

C. Sumpownon: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
P. Sobhon: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.
A. Engsusophon and N. Kornthong: Performed the experiments.
C. Chotiwitthanakun and P. Meeratana: Contributed reagents, materials, analysis tools or data.

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Data availability statement
Data will be made available on request.

Declaration of interests statement
The authors declare no conflict of interest.

Additional information
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