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RESEARCH ARTICLE

Prostaglandin catabolism in *Spodoptera exigua*, a lepidopteran insect

Shabbir Ahmed and Yonggyun Kim*

ABSTRACT

Several prostaglandins (PGs) and PG-synthesizing enzymes have been identified from insects. PGs mediate cellular and humoral immune responses. However, uncontrolled and prolonged immune responses might have adverse effects on survival. PG catabolism in insects has not been reported. Here, using a transcriptomic analysis, we predicted the presence of two PG-degrading enzymes, *PG dehydrogenase* (*SePGDH*) and *PG reductase* (*SePGR*), in *Spodoptera exigua*, a lepidopteran insect. *SePGDH* and *SePGR* expression levels were upregulated after immune challenge. However, their expression peaks occurred after those of PG biosynthesis genes, such as those encoding PGE_2 synthase or PGD_2 synthase. *SePGDH* and *SePGR* expression levels were upregulated after injection with PGE_2 or PGD_2. In contrast, such upregulated expression was not detected after injection with leukotriene B_4, an eicosanoid inflammatory mediator. RNA interference (RNAi) using double-stranded RNAs specific to *SePGDH* or *SePGR* suppressed their expression levels. The RNAi treatment resulted in an excessive and fatal melanization of larvae even after a non-pathogenic bacterial infection. Phenoloxidase (PO) activity mediating the melanization in larval plasma was induced by bacterial challenge or PGE_2 injection. Although the induced PO activity decreased after 8 h in control larvae, those treated with dsRNAs specific to PG-degrading enzyme genes kept a high PO activity for a longer period. These results suggest that SePGDH and SePGR are responsible for PG degradation at a late phase of the immune response.

KEY WORDS: Eicosanoids, RNA interference, Immunity

INTRODUCTION

Prostaglandins (PGs) are a group of eicosanoids derived from arachidonic acid (AA) by catalysis of cyclooxygenase (COX). PGs can act as autocrine and paracrine signals and mediate various physiological processes such as reproduction, immunity and thermal homeostasis in mammals (Gilroy and Bishop-Bailey, 2019). In insects, PGs are the first known eicosanoid signal molecules that can influence oocyte development, egg-laying behavior and immunity (Stanley and Kim, 2019).

PG biosynthesis pathways in insects are likely to be similar to those in mammals (Scarpati et al., 2019). However, at least two steps are unique in insects. One is the step to obtain AA because most terrestrial insects appear to possess trace amounts of AA in their phospholipids (PLs) (Stanley and Kim, 2019). Thus, insects should have an alternative strategy to provide substrates for PG biosynthesis. Relatively high linoleic acid (LA) content in insect PLs suggested that LA is released by phospholipase A_2 (PLA_2) and subsequently elongated/desaturated to AA (Hasan et al., 2019). Another unique step of PG biosynthesis in insects is the catalysis by a peroxynectin (Pxt) because there are no COX orthologs in insect genomes (Varvas et al., 2009). Indeed, it has been demonstrated in some insects that Pxts catalyze the oxygenation of AA to PGH_2 (Tootle and Spradling, 2008; Park et al., 2014). PGH_2 is a common substrate in the formation of various PGs including prostanoids and prostacyclins. For example, PGE_2 synthase (PGES) and PGD_2 synthase (PGDS) have been identified in beet armyworm, *Spodoptera exigua*, in which PGE_2 and PGD_2 can mediate immune and reproductive processes (Ahmed et al., 2018; Sajjadian et al., 2020). In particular, PGE_2 can stimulate melanization during cellular immune responses such as hemocytic nodulation and encapsulation by mediating the release of prophenoloxidase (PPO) from its synthetic hemocytes called oenocytoids (Shrestha and Kim, 2008). Released PPO is then activated by a proteolytic cleavage of PPO-activating protease via a cascade of activating serine proteases (Jiang et al., 2010). However, uncontrolled and excessive PGs might fatally damage an insect's tissues. To avoid this kind of self-intoxication, PG levels should be tightly controlled between production and degradation (Ahmed et al., 2019). Unlike PG biosynthesis, PG degradation pathways are not known in insects.

In mammals, PGs are short-lived mediators degraded by oxidation (Tai et al., 2002). PG dehydrogenase (PGDH) is responsible for PG metabolism by catalyzing NAD^+-linked oxidation of 15(S)-hydroxyl group of PGs and lipoxins to yield inactive 15-keto metabolites. These metabolites are further degraded by NADPH/NADH-dependent 15-oxoprostaglandin-Δ^{13}-reductase (PGR). The objective of the present study was to identify *PGDH* and *PGR* orthologs from the *S. exigua* genome to investigate PG metabolism in insects. To validate functional associations of PGs with their biological activities, this study used an RNA interference (RNAi) approach. The results demonstrate that reduced PG degradation by RNAi silencing of *PGDH* or *PGR* expression has fatal consequences.

MATERIALS AND METHODS

Insect rearing and bacterial culture

Larvae of *Spodoptera exigua* (Hübner 1808) were reared on an artificial diet (Goh et al., 1990) at a temperature of 25±2°C and relative humidity of 60±5% with a photoperiod of 16 h:8 h (light:dark). The artificial diet was prepared according to an earlier study (Shrestha et al., 2011). Adults were provided with 10% sucrose for oviposition. Under these rearing conditions, *S. exigua* underwent five larval instars (L1–L5) before pupation. *Escherichia coli* Top10, a Gram-negative bacterium (Invitrogen, Carlsbad, CA, USA) was cultured overnight in Luria-Bertani (LB) medium at 37°C. For

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immune challenge, these bacteria were heat-killed at 95°C for 10 min. Bacterial cells were counted with a hemocytometer (Neubauer improved bright-line, Superior Marienfeld, Lauda-Königshofen, Germany) under a phase contrast microscope (BX41, Olympus, Tokyo, Japan). Bacterial suspensions were diluted with sterilized and deionized distilled H_2O for preparing treatment dose (4.1×10^4 cells μl^{-1}).

Chemicals

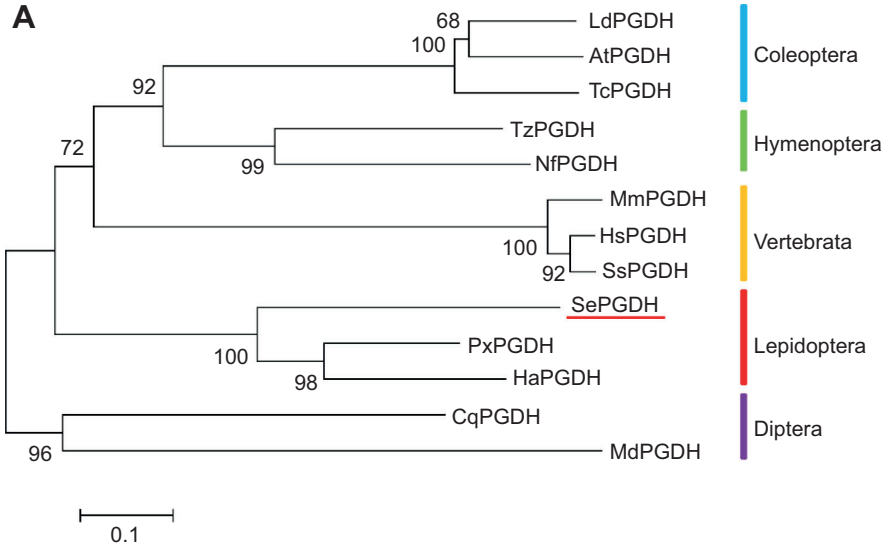
Prostaglandin D_2 (PGD_2: 9α,15S-dihydroxy-11-oxo-prosta-5Z,13E-dien-1-oic acid), prostaglandin E_2 (PGE_2: 9-oxo-11α,15S-dihydroxy-prosta-5Z,13E-dien-1-oic acid) and leukotriene B4 (LTB_4: 5S,12R-dihydroxy-6Z,8E,10E,14Z-eicosatetraenoic acid) were purchased from Cayman Chemical (Ann Arbor, MI, USA). These chemicals were all dissolved in dimethyl sulfoxide (DMSO).

Bioinformatics analysis

DNA sequences of *S. exigua* PGDH (SePGDH) and SePGR were obtained from the Transcriptome Shotgun Assembly (TSA) database deposited at NCBI GenBank with accession numbers GAOQ01017731.1 and GAOQ01013314.1, respectively. Prediction of protein domain structure was performed using Pfam (http://pfam.xfam.org) and Prosite (https://prosite.expasy.org/). Phylogenetic analysis and phylogenetic tree construction with the neighbor-joining method were performed using MEGA 6.0 and ClustalW programs. Bootstrapping values were obtained with 1500 repetitions to support branches and clustering.

RT-qPCR

RNA extraction and cDNA preparation followed the method described by Ahmed et al. (2018). Melting curves of products were obtained to confirm amplification specificity. Quantitative analysis was done using a comparative CT method (Livak and Schmittgen, 2001) to estimate the relative mRNA expression level of a target gene compared with that of *RL32*, a ribosomal gene, as an internal control (Park et al., 2015). To determine expression levels of *SePGDH*, *SePGR*, *SePGES* and *SePGDS* after bacterial challenge, heat-killed *E. coli* was injected into L5 larvae at a dose of 4.1×10^4 cells per larva. Expression levels were also checked after injecting 1 µg of PGE_2, PGD_2 or LTB_4 per



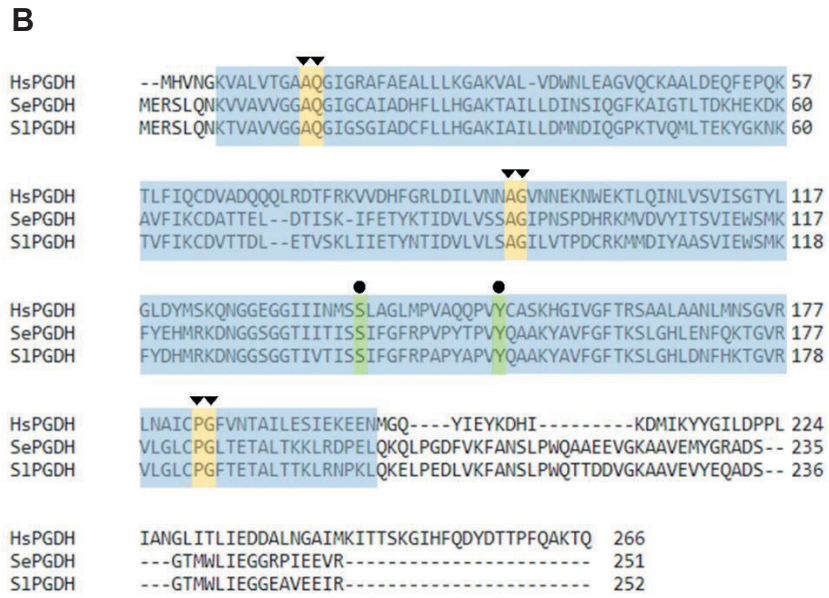


Fig. 1. Molecular characterization of prostaglandin (PG) dehydrogenase of *Spodoptera exigua* (*SePGDH*). (A) Phylogenetic analysis of the predicted amino acid sequences of insect and vertebrate PG dehydrogenases (*PGDH*s). The analysis was performed using MEGA 6. Bootstrapping values were obtained with 1500 repetitions to support branches and clustering. Species acronyms and GenBank accession numbers are shown in Table S2. (B) Multiple sequence alignment of SePGDH with PGDHs of *Spodoptera litura* (XP_022830962.1) and *Homo sapiens* (NP_000851.2). The blue region denotes a short-chain dehydrogenase/reductase family domain. Dots and triangles above residues represent active site and core residues for NAD^+ binding, respectively. Protein domains were predicted using Pfam (http://pfam.xfam.org) and Prosite (https://prosite.expasy.org/).

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larva into L5 larvae. The experiment was independently replicated 3 times. All primer sequences used in this study are represented in Table S1.

RNAi

RNAi was performed using gene-specific dsRNA prepared using a MEGAscript RNAi kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. *SePGDH* (287 bp) and *SePGR* (236 bp) DNA fragments were obtained by PCR using gene-specific primers (Table S1) containing the T7 promoter sequence at the 5′ end. Sense and antisense RNA strands were synthesized using T7 RNA polymerase at 37°C for 4 h. The resulting dsRNA was mixed with the transfection reagent Metafectene PRO (Biontex, Plannegg, Germany) at 1:1 (v/v) ratio and then incubated at 25°C for 30 min to form liposomes. A 1 µg sample of dsRNA was injected to larval hemocoel using a microsyringe (Hamilton, Reno, NV, USA) equipped with a 26-gauge needle. At 24 h post-injection, RNAi efficacy was determined by RT-qPCR as described above. Control dsRNA (dsCON) specific for a green fluorescent protein (GFP) gene (Vatanparast et al., 2018) was also prepared. Each RNAi treatment was replicated 3 times using independent RNA samples.

Phenoloxidase assay

Plasma phenoloxidase (PO) activity was determined using L-3,4-dihydroxyphenylalanine (DOPA) as a substrate. Each L5 larva

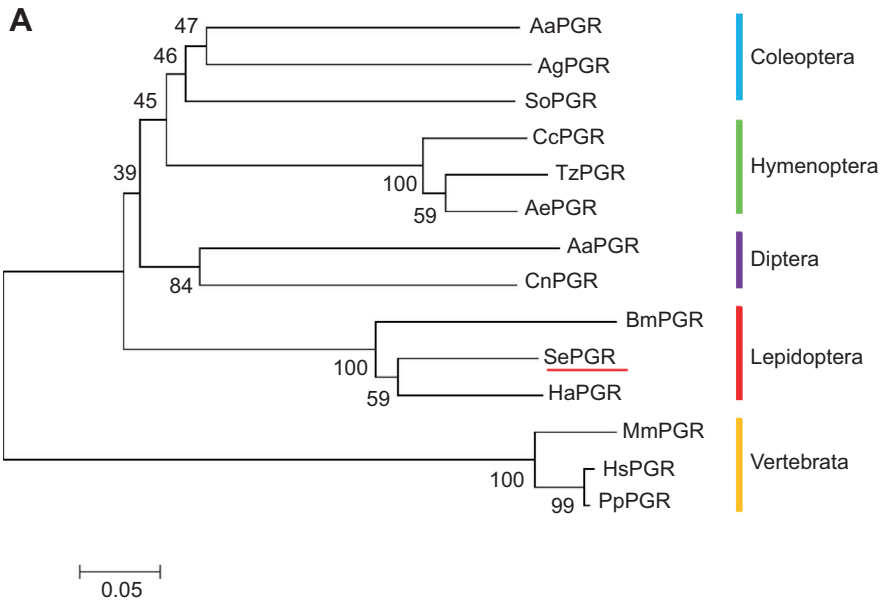

Fig. 2. Molecular characterization of PG reductase of *S. exigua* (*SePGR*). (A) Phylogenetic analysis of the predicted amino acid sequences of insect and vertebrate *PGR*s. The analysis was performed using MEGA 6. Bootstrapping values were obtained with 1500 repetitions to support branches and clustering. Species acronyms and GenBank accession numbers are shown in Table S2. (B) Multiple sequence alignment of SePGR with PGRs of *S. litura* (XP_022825289.1) and *H. sapiens* (AAH35228.1). Green and blue regions denote oxidoreductase and $NADP^+$-binding domains, respectively. Dots and triangles above residues represent active site and core residues for $NADP^+$ binding, respectively. Protein domains were predicted using Pfam (http://pfam.xfam.org) and Prosite (https://prosite.expasy.org/).

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was injected with 1 µl of different concentrations of PGE_2 or heat-killed *E. coli* (4.1×10^4 cells per larva^{-1}). At 24 h post-injection of dsRNA (1 µg larva^{-1}), 500 µl of hemolymph was collected from ∼10 larvae treated with heat-killed *E. coli* into a 1.7 ml tube at different time points. Hemolymph was centrifuged at 800 ***g*** for 5 min at 4°C to collect supernatant (plasma fraction). The total reaction volume was 200 µl, consisting of 180 µl of 10 mmol l^{-1} DOPA in PBS and 20 µl of the plasma sample. Absorbance (*A*) was read at 495 nm using a VICTOR multi label plate reader (PerkinElmer, Waltham, MA, USA). PO activity was expressed as ΔA min^{-1} µl^{-1} plasma. Each treatment consisted of three biologically independent replicates.

Effect of dsRNAs specific to PG degradation-associated genes on larval mortality

A 1 µg sample of dsPGDH or dsPGR was injected subcutaneously into 1 day old L5 larva. At 24 h post-injection, live *E. coli* cells were injected at a dose of 4.1×10^4 cells per larva. Mortality was assessed 20 h after bacterial treatment. Each treatment was replicated 3 times. Each replication used 10 individuals.

Statistical analysis

Data from all assays were subjected to one-way analysis of variance (ANOVA) using PROC GLM (SAS Institute Inc., 1989) for continuous variables. All data (means±s.d.) were plotted using Sigma plot. Means were compared with a least squared difference (LSD) and discriminated at a Type I error of 0.05. Significance of the difference between two groups was tested using a *t*-test (Sigma plot version 12.0). $P<0.05$ was considered statistically significant for *t*-tests.

RESULTS

PG degradation enzymes

Using *PGDH* (GenBank accession number: XP_022830962.1) and *PGR* (XP_022825289.1) of *S. litura*, corresponding orthologs (*SePGDH* and *SePGR*) were obtained from transcriptome databases (GAOQ01017731.1 and GAOQ01013314.1, respectively) of *S. exigua*. *SePGDH* and *SePGR* and encoded 251 and 337 amino acid sequences, respectively. The predicted amino acid sequence of *SePGDH* shared 40–77% sequence similarity with other lepidopteran PGDHs. Phylogenetic analysis showed that it formed

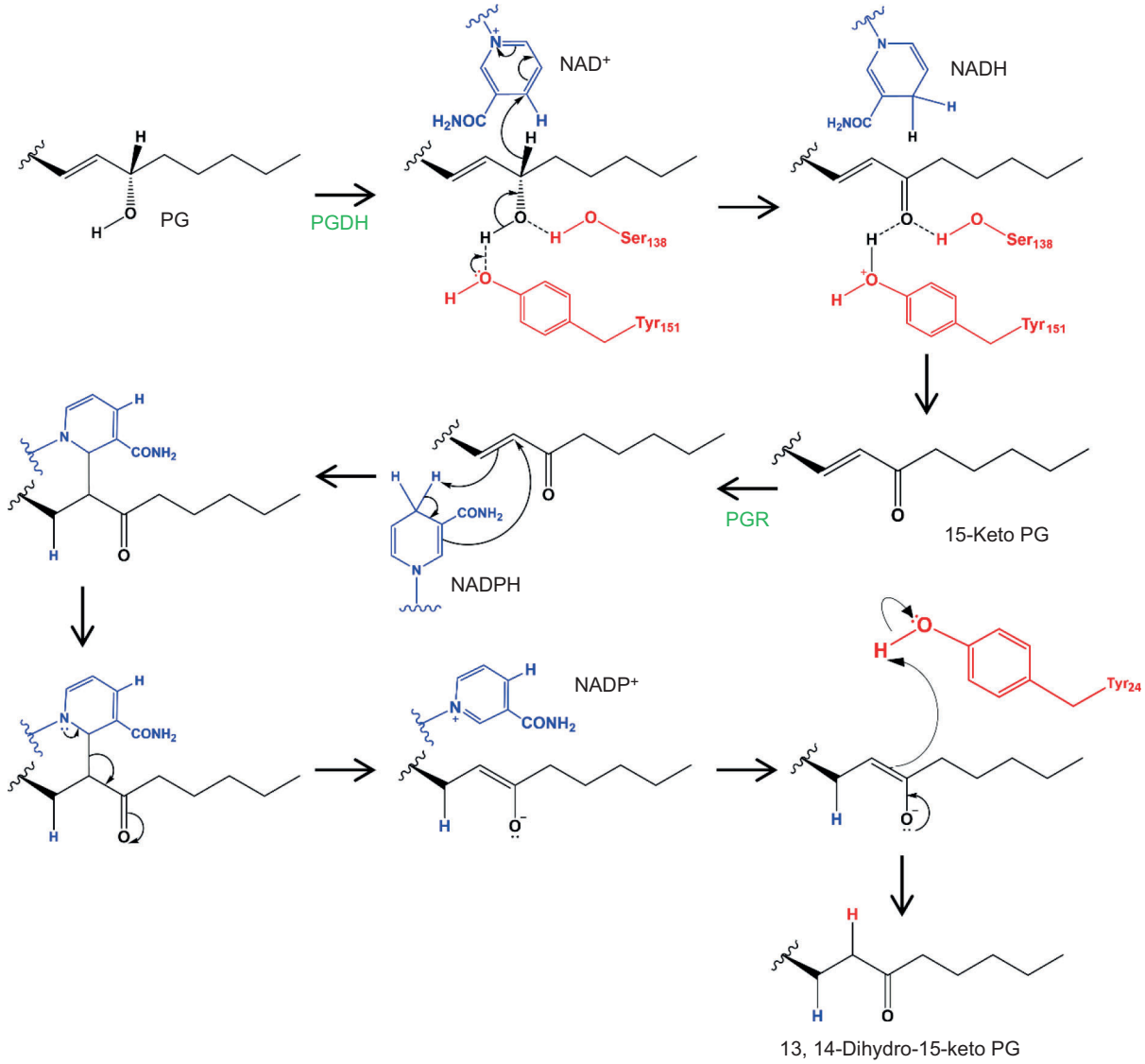

Fig. 3. Diagram of the catalytic steps of PG degradation in *S. exigua*. PG degradation is catalyzed by the enzymes PGDH and PGR.

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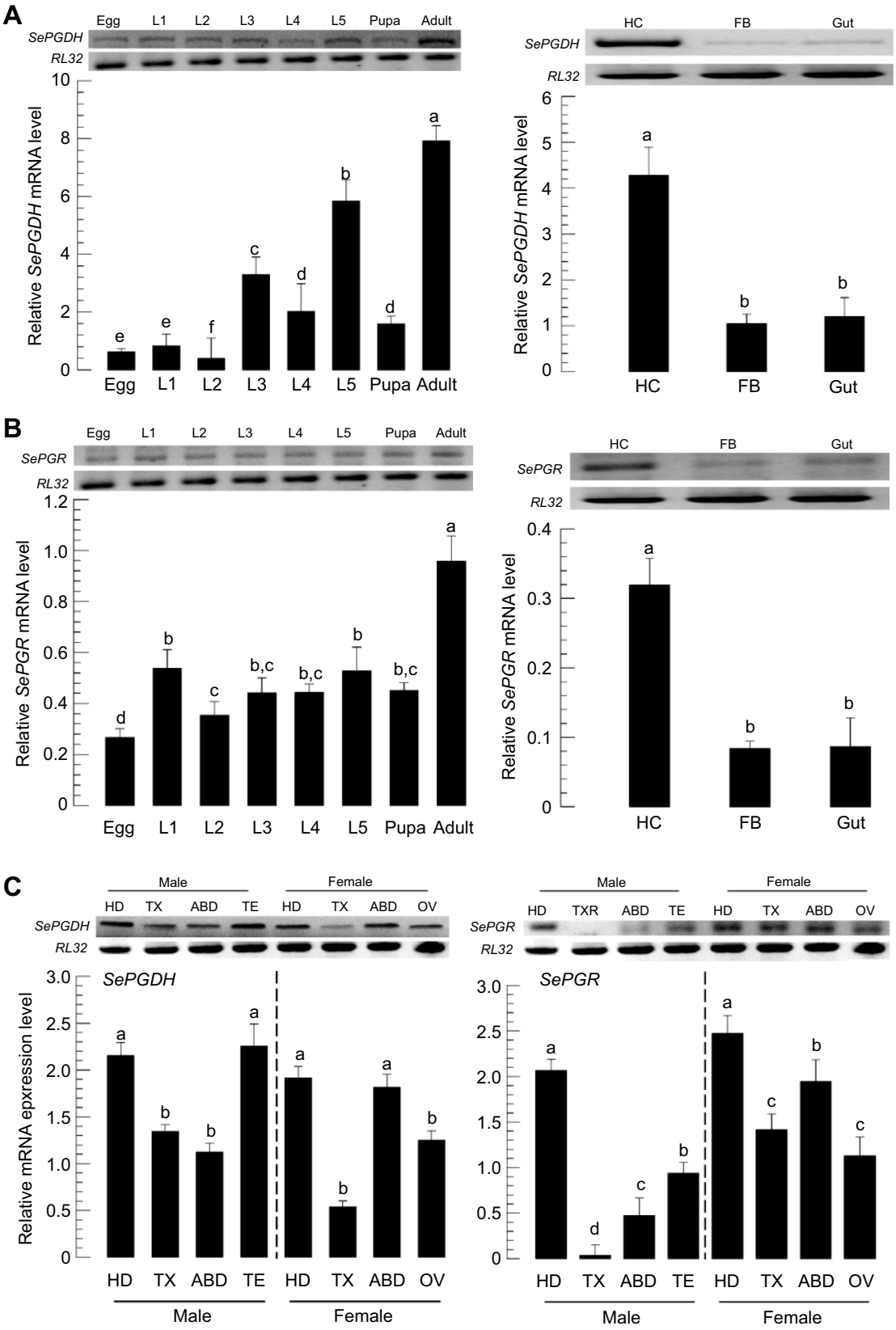

Fig. 4. Expression profiles of PG degradation-associated genes. (A,B) *SePGDH* (A) and *SePGR* (B) expression levels in different developmental stages [left: egg, first to fifth instar larvae (L1–L5), pupa and adult] and different tissues [right: hemocyte (HC), fat body (FB) and midgut (Gut)] of *S. exigua* larvae. (C) Expression patterns of *SePGDH* and *SePGR* in different body parts (HD, head; TX, thorax; ABD, abdomen; TE, testis; and OV, ovary) of adults. Each treatment was replicated 3 times. A ribosomal gene, *RL32*, was used as an internal control. Different letters indicate significant differences among means at Type I error=0.05 (LSD test). Representative agarose gels of PCR products are shown above the graphs.

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a monophyletic cluster with lepidopteran PGDHs, separated from orthologs of other insects or vertebrates (Fig. 1A). Interestingly, hymenopteran and coleopteran PGDHs are more closely related to vertebrate PGDHs than to lepidopteran and dipteran PGDHs. This suggests that PGDHs are highly conserved among metazoans. In addition to NAD-binding regions, serine (S138) and tyrosine (Y151) at the catalytic site were conserved (Fig. 1B). The predicted *SePGR* amino acid sequence shared 55–92% sequence similarity with other lepidopteran PGRs. It formed a monophyletic cluster with lepidopteran PGRs in phylogenetic analysis (Fig. 2A). In addition to NADPH-binding regions, tyrosine (Y245) at the catalytic site was conserved (Fig. 2B). These conserved sites of

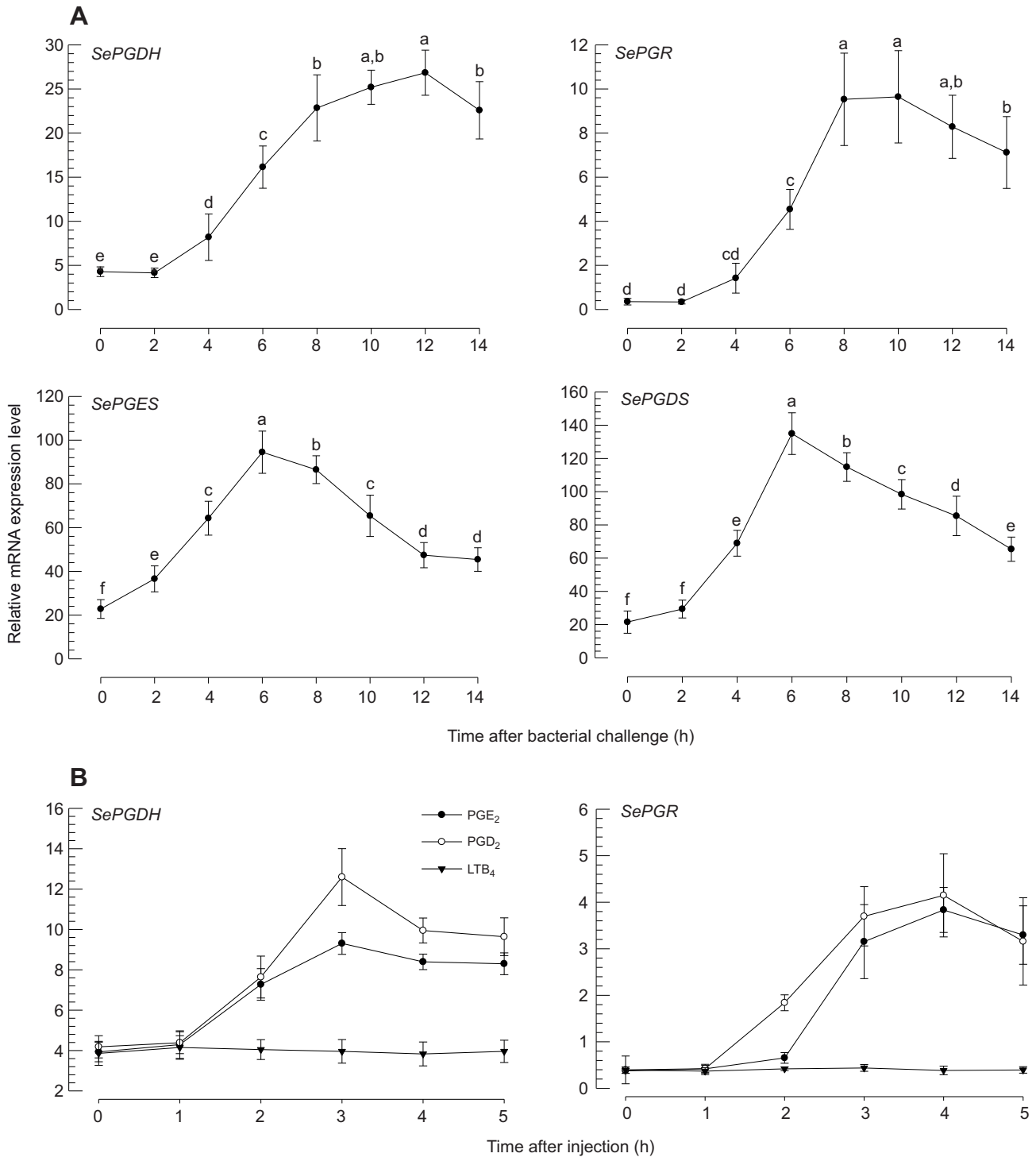

Fig. 5. Induction of *SePGDH* or *SePGR* expression after bacterial challenge or eicosanoid injection. (A) Expression of *SePGDH*, *SePGR*, *SePGES* and *SePGDS* following bacterial challenge. Expression levels were detected after injecting heat-killed *E. coli* at a dose of 4.1×10^4 cells per larva into L5 larvae. (B) Expression of *SePGDH* and *SePGR* following injection of prostaglandin E_2 (PGE_2) and D_2 (PGD_2) or the eicosanoid inflammatory mediator LTB_4. Expression levels were checked after injecting 1 µg per larva of PGE_2, PGD_2 or LTB_4 into L5 larvae. Each treatment was replicated 3 times. A ribosomal gene, *RL32*, was used as an internal control. Different letters indicate significant differences among means at Type I error=0.05 (LSD test).

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SePGDH and SePGR suggest a degradation pathway of PGs into 13,14-dihydro-15-keto PGs (Fig. 3). In this model, SePGDH catalyzes the action of PGE_2 or PGD_2 to form 15-keto-PGs. These 15-keto-PGs are then converted to 13,14-dihydro-15-keto PGs by SePGR.

Expression profile of *SePGDH* and *SePGR*

SePGDH and *SePGR* were expressed in all developmental stages (from egg to adult) of *S. exigua* (Fig. 4), although there were significant ($P<0.05$) differences in their expression levels among stages, with adults having the highest expression levels. Expression levels increased with larval development for *SePGDH* (Fig. 4A) but not for *SePGR* (Fig. 4B). Both genes were highly expressed in hemocytes. In adults, *SePGDH* and *SePGR* were highly expressed in male and female heads (Fig. 4C). Both reproductive organs (testis in male and ovary in female) showed high expression levels of *SePGDH* and *SePGR*.

When L5 larvae were immune challenged with heat-killed *E. coli*, *SePGDH* and *SePGR* expression levels in hemocytes were significantly up-regulated (Fig. 5A). Such bacterial challenge also increased expression levels of PG synthesis-associated genes such as *SePGES* and *SePGDS*. However, there was a difference in the induction pattern between PG degradation-association genes and PG synthesis-associated genes. *SePDGH* expression was up-regulated at 4 h post-injection, reaching the highest level at 10 h post-injection. *SePGR* expression was up-regulated at 6 h post-injection, reaching the highest level at 8 h post-injection. In contrast, both PG synthesis-associated genes showed rapid increases at 2 h post-injection, peaking at 6 h post-injection followed by rapid decreases of their expression levels. Thus, expression of PG synthesis-associated genes preceded that of PG degradation-associated genes.

We then analyzed expression patterns of *SePGDH* and *SePGR* in response to increased levels of PGs (Fig. 5B). Either PGE_2 or PGD_2 injection significantly increased expression levels of both genes as early as 2 h post-injection. However, injection with LTB_4, an eicosanoid inflammatory mediator, did not change the expression level of *SePDGH* or *SePGR*.

RNAi of PG degradation-associated genes leads to increased immune response

After injection of gene-specific dsRNA, expression of *SePGDH* (Fig. 6A) and *SePGR* (Fig. 6B) was significantly ($P<0.05$) suppressed. RNAi caused more than 60% reduction of expression in hemocytes at 24 h after dsRNA injection (Fig. 6). RNAi effects were also observed in fat body and midgut tissues, although *SePGDH* and *SePGR* were expressed at much lower levels in these tissues.

PO activity in L5 larvae was significantly ($P<0.05$) increased after PGE_2 injection and this induced activity was dependent on PGE_2 concentration (Fig. 7A). PO activity was then investigated at different time points after bacterial challenge (Fig. 7B). Control larvae showed the highest PO activity at 8 h post-injection followed by a rapid decrease in activity. However, larvae treated with dsRNA specific to either *SePGDH* or *SePGR* showed an increase in PO activity without a subsequent decrease at 8 h post-injection. Later, some RNAi-treated larvae became darken due to excessive melanization and finally died in response to the non-pathogenic bacterial infection (Fig. 7C). Larval mortality at 20 h post-injection was significantly higher in the RNAi-treated group (dsRNA specific to *SePGDH* or *SePGR*) than in the control group.

DISCUSSION

PGs play crucial roles in mediating various physiological processes in insects (Stanley and Kim, 2019). In *S. exigua*, these include PO activation (Shrestha and Kim, 2008), egg-laying behavior (Ahmed et al., 2018), oocyte development (Al Baki and Kim, 2019) and various immune responses (Kim et al., 2018). Furthermore, biosynthetic pathways from PGH_2 to PGE_2 by catalytic activity of SePGES and from PGH_2 to PGD_2 by catalytic activity of SePGDS are known in *S. exigua* (Ahmed et al., 2018; Sajjadian et al., 2020). However, genetic factors involved in PG degradation in insects

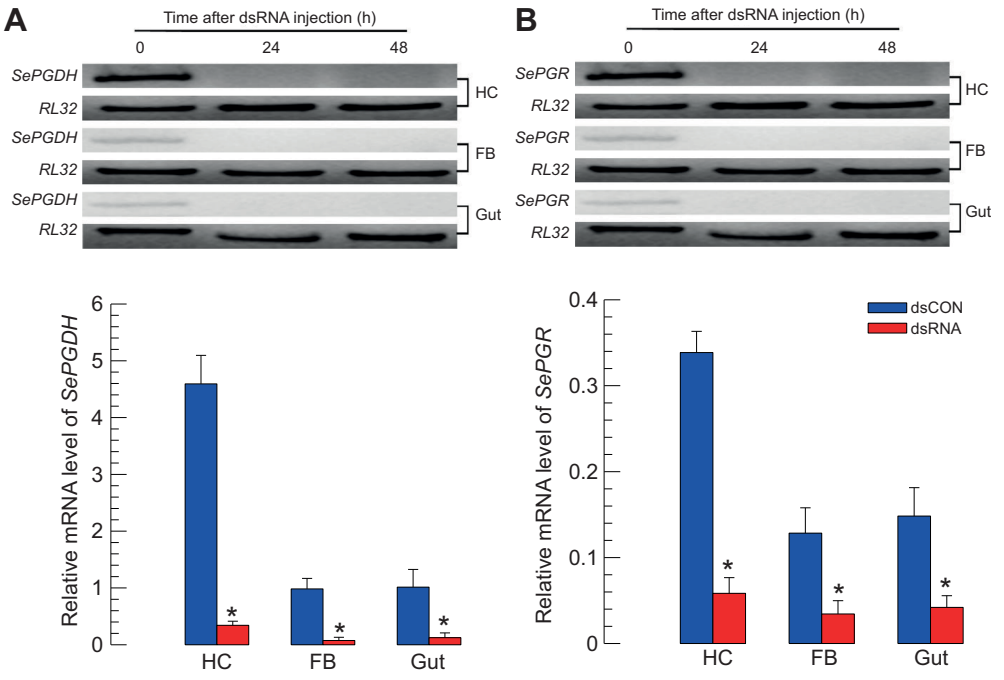

Fig. 6. RNA interference (RNAi) of *SePGDH* or *SePGR* expression. Effect of RNAi on *SePGDH* (A) and *SePGR* (B) expression in different tissues (HC, hemocyte; FB, fat body; Gut, midgut) of L5 larvae of *S. exigua*. dsRNA (dsCON) specific for the green fluorescent protein (*GFP*) gene was used as a control. qPCR data were analyzed at 24 h post-injection. Each treatment was replicated 3 times. A ribosomal gene, *RL32*, was used as an internal control. Asterisks indicate significant differences among means at Type I error=0.05 (LSD test).

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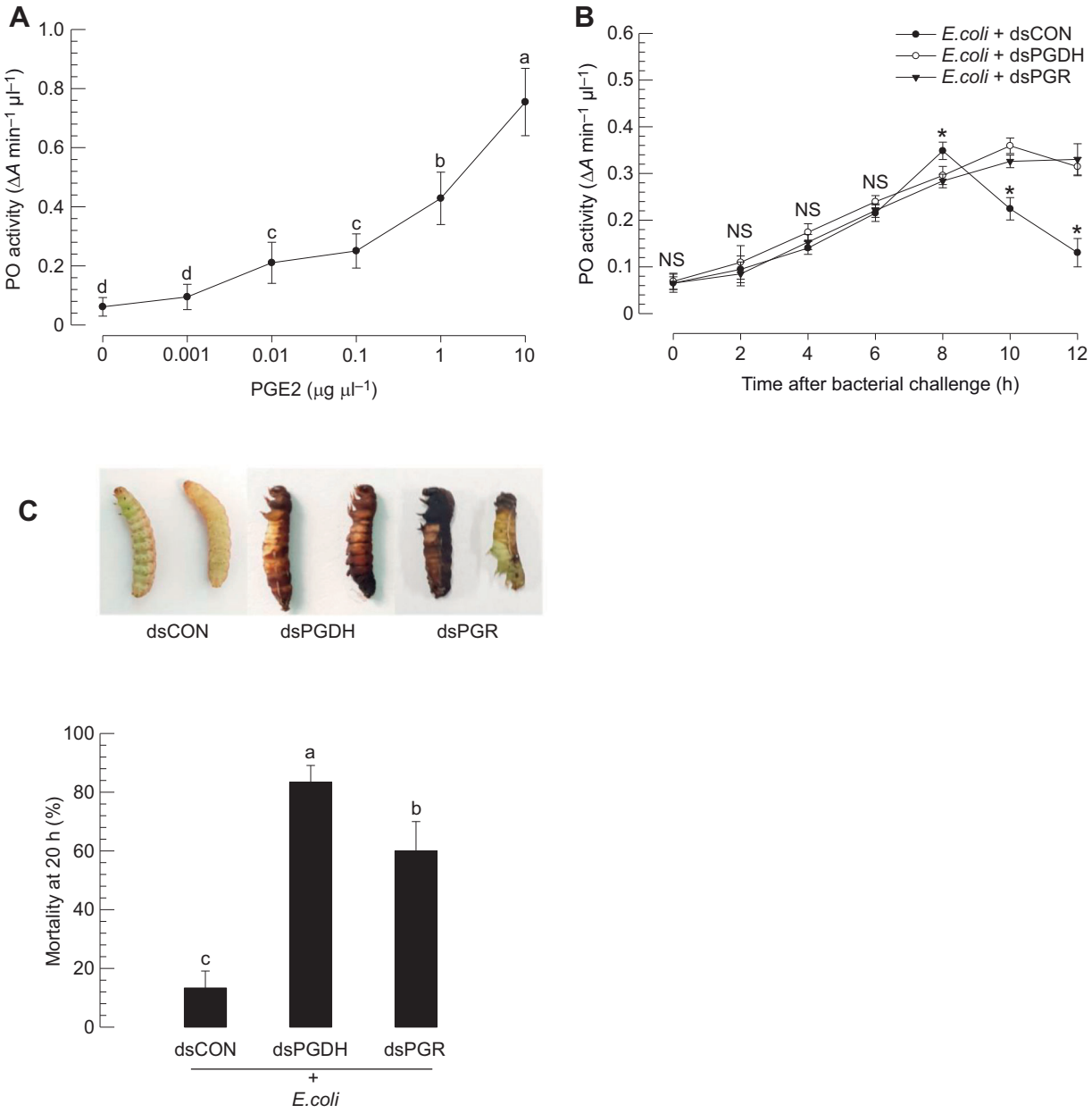

Fig. 7. Increased immunity induced by RNAi against *SePGDH* or *SePGR* expression in *S. exigua*. (A) Induced phenoloxidase (PO) activity after PGE_2 injection (measured as change in absorbance, ΔA). PGE_2 (1 µl) at different concentrations was injected into each L5 larva. (B) Prolonged activity of PO in response to dsRNA. dsRNA (1 µg) specific to *SePGDH* or *SePGR* (or control dsCON) was injected into each L5 larva. At 24 h post-injection, heat-killed *E. coli* cells were injected into L5 larvae at a dose of 4.1×10^4 cells per larva. (C) Mortality after injection of dsRNA. At 24 h post-injection of the dsRNA treatment, live *E. coli* cells were administered subcutaneously. Mortality was assessed at 20 h after bacterial treatment. Each treatment was independently replicated 3 times. Different letters indicate significant differences among means at Type I error=0.05 (LSD test).

including *S. exigua* were not known. This study reports two PG degradation-associated enzymes in *S. exigua*.

Identification of these two PG degradation-associated genes (*SePGDH* and *SePGR*) was supported by bioinformatics analysis. The predicted amino acid sequence of SePGDH indicated a conserved catalytic triad (Gln148, Tyr151 and Asn95) in the active site (Al-Najjar, 2018). Tyr151 plays a crucial role in linking enzyme and substrate with a hydrogen bond. SePGR has a conserved Tyr residue near the Src homology domain. It may behave like the catalytic Tyr245 and Tyr259 of vertebrate PGR-1 and PGR-2, respectively (Chou et al., 2007). This Tyr residue participates in the hydrogen bond network around the 2′-hydroxyl group of nicotine amide ribose, which interacts with two water molecules to stabilize an enolate intermediate for the catalysis of 15-keto-PGE_2 reduction (Hori et al., 2004). This prediction also proposes a PG degradation pathway from PGs to 15-keto-PGs by SePGDH and from 15-keto-PGs to 13,14-dihydro-15-keto-PGs by SePGR based on a vertebrate model (Robinson et al., 1989). In general, 15-keto-PGE_2 has been regarded as an inactive form. However, it is active in stimulating the egg-laying behavior of a cricket, *Teleogryllus commodus*, probably by binding to an as-yet unidentified receptor (Stanley-Samuelson et al., 1986). Recent studies in mammals have also shown that 15-keto-PGE_2 can mediate biological functions as an endogenous ligand for peroxisome proliferator-activated

receptor γ (PPAR-γ), as demonstrated in pathogenesis of cystic fibrosis in a mouse model, which is associated with regulation of PPAR-γ by 15-PGDH-derived 15-keto-PGE_2 (Harmon et al., 2010). In hepatocellular cancer cells, 15-keto-PGE_2 can activate PPAR-γ and regulate its downstream genes (Lu et al., 2014). Our current study showed that a specific RNAi against PGR expression failed to prevent PG's action against PO activation in *S. exigua*. This suggests that 15-keto-PG, which might be accumulated after RNAi treatment, is not effective in mediating PO activation in *S. exigua*.

Both *SePGDH* and *SePGR* were highly expressed in larval and adult stages of *S. exigua*. In adults, these two genes need to be expressed because PGs play crucial roles in mediating reproductive processes of *S. exigua* (Ahmed et al., 2018). In larvae, the genes were highly expressed in hemocytes. The bacterial challenge enhanced their expression in hemocytes. However, their peak expression occurred after the maximal expression of PG synthesis-associated genes such as *SePGES* and *SePGDS*. This suggests that PGs produced by the catalytic activity of SePGES and SePGDS might stimulate the expression of *SePGDH* and *SePGR*. This was supported by the significant induction of gene expression after PGE_2 or PGD_2 injection. Gene induction was not observed after injection of LTB_4. The PGE_2 receptor of *S. exigua* is known to use cAMP to activate downstream signals (Kim et al., 2020), suggesting that an immune challenge can induce the expression of *SePGES* and *SePGDS* as a result of up-regulated PG levels via the cAMP signaling pathway. Induction of PGDH expression by cAMP is supported by the presence of cAMP-responsive element-binding protein on its promoter (Greenland et al., 2000). The expression of *SePGDH* and *SePGR* in response to the cAMP signal needs to be explored. In mammalian white blood cells, protein kinase C can also activate PGDH synthesis and activity (Xun et al., 1991). These findings suggest that the expression of *SePGDH* and *SePGR* might be influenced by several factors other than PGs in *S. exigua*.

RNAi of *SePGDH* or *SePGR* expression impaired the control of PO activation in response to bacterial challenge. Upon immune challenge, PO activity was increased for 8 h. It was then decreased to avoid unnecessary immune responses. Here, PGE_2 is involved in mediating PO activation by inducing oenocytoid cell lysis to release PPO (Shrestha and Kim, 2008). Cell lysis is mediated by PGE_2 through a membrane receptor, which activates a sodium–potassium–chloride cotransporter to generate an ion gradient for cell rupture (Shrestha et al., 2011, 2015). Released PPO is then activated by a cascade of serine proteases (Jiang et al., 2010), resulting in a dose-dependent PO activation by PGE_2. This was confirmed in our current study. RNAi of PG degradation genes failed to break down PGs, leading to a prolonged activation of PO. Excess PO activity resulted in uncontrolled and fatal melanization. Interestingly, there was a significant difference in insect mortality between the two RNAi treatments against *SePGDS* and *SePGR* expression. This difference could be a functional SePGDH catalytic activity in RNAi specific to *SePGR*, which might lead to reduced insect mortality than with the RNAi specific to *SePGDH*. This supports the role of SePGDS and SePGR in degrading PGs in *S. exigua*. Thus, this study reports the first PG degradation pathway in insects by identifying *SePGDH* and *SePGR* in *S. exigua*. Subsequent studies need to demonstrate that the gene products degrade active PGs through a biochemical analysis.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: Y.K.; Methodology: S.A., Y.K.; Software: S.A.; Validation: S.A.; Formal analysis: S.A.; Investigation: S.A., Y.K.; Resources: Y.K.; Data curation: S.A.; Writing - original draft: S.A.; Writing - review & editing: Y.K.; Visualization: S.A.; Supervision: Y.K.; Project administration: Y.K.; Funding acquisition: Y.K.

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