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Short communication

A multidomain galectin involved in innate immune response of pearl oyster *Pinctada fucata*

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ABSTRACT

Galectins could specifically bind to β-galactoside residues and play crucial roles in innate immune responses of vertebrates and invertebrates. In this study, the cDNA of a galectin with multiple carbohydrate-recognition domains (CRDs) was cloned from pearl oyster *Pinctada fucata* (designated as PoGal). PoGal cDNA was 2138 bp long and consisted of a 5′-untranslated region (UTR) of 120 bp, a 3′-UTR of 350 bp with two cytokine RNA instability motifs (ATTTA), and an open reading frame (ORF) of 1668 bp encoding a polypeptide of 555 amino acids with an estimated molecular mass of 63.4 kDa and a theoretical isoelectric point of 4.8. PoGal contained four CRDs, each CRD of PoGal all had the conserved carbohydrate-binding motifs H-NPR and WG-ER. PoGal shared 43.7% and 62.9% identity to those of bay scallop and eastern oyster, respectively, which were only two galectins with four CRDs. The phylogenetic analysis revealed that all galectins with four CRDs formed a single clade. PoGal mRNA was constitutively expressed in all detected tissues, and the expression level of PoGal mRNA was significantly up-regulated in digestive gland, mantle, haemocyte, gonad and intestine after *Vibrio alginolyticus* stimulation. The expression profile analysis showed that the expression level of PoGal mRNA was significantly up-regulated at 4, 8 and 12 h after *V. alginolyticus* stimulation. These results suggested that PoGal was a constitutive and inducible acute-phase protein that perhaps involved in innate immune response of pearl oyster.

1. Introduction

Galectins are a family of β-galactoside-binding lectins that were first found in the electric organ of the electric eel in 1975 (Teichberg et al., 1975; Barondes et al., 1994a) and then identified in a wide range of multicellular organisms from fungi to mammals (Barondes et al., 1994b; Cooper et al., 1997; Wada and Kanwar, 1997; Nemoto-Sasaki et al., 2008). Galectins have at least one conserved carbohydrate-recognition domain (CRD), which can bind to glycans linked to various cell surface receptors, and then regulate a variety of biological processes in vertebrates, such as cell adhesion (van den Brûle et al., 1995; Friedrichs et al., 2007; Alge-Priglinger et al., 2009), development (Kim et al., 2009; de Boer et al., 2009), cancer progression (Cludts et al., 2009; Canesin et al., 2010), cytokine secretion (Rabinovich et al., 1999; Filer et al., 2009), apoptosis (Sturm et al., 2004; Koh et al., 2009) and immune regulation (Ferraz et al., 2008; Mengshol et al., 2010).

In invertebrates, the recent studies indicated that host galectins could functions as pattern-recognition receptors (PRRs) that targeted glycans on the surfaces of viruses, bacteria and parasites (Rabinovich and Gruppi, 2005; Sato et al., 2009). The galectin of eastern oyster *Crassostrea virginica* (CvGal) is responsible for recognizing the protozoan parasite *Perkinsus marinus* (Tasumi and Vasta, 2007). A tandem-repeat galectin, McGal from Manila clam *Ruditapes philippinarum* has also been proved to bind to the surface of *Perkinsus olseni* as PRR, and is up-regulated after challenge with *P. olseni* or *Vibrio tapetis* (Kim et al., 2008). Another tandem-repeat galectin, BgGal from freshwater snail *Biomphalaria glabrata* selectively recognizes the schistosome-related sugar, lacNAc, and strongly binds to haemocytes and the tegument of *Schistosoma mansoni* sporocysts in a sugar-inhibitable fashion (Yoshino et al., 2008). A multidomain galectin, AiGal from bay scollap *Argopecten irradians* was also characterized and involved in the innate immune responses (Song et al., 2010).

In contrast with the abundant knowledge on galectins in vertebrates, the information of galectins in bivalve mollusk is still poorly known. To further understand the molecular evolution and functions of galectins in the innate immune response of invertebrates, in this study, we cloned and characterized a multidomain galectin

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from pearl oyster *Pinctada fucata* (designated as PoGal), which is the most important farmed bivalve mollusk for seawater pearl production in China (Zhang et al., 2009, 2010), and investigated its tissue distribution and temporal expression profile after bacterial challenge. The work would hopefully provide insight into evolution of multidomain galectins as well as its important functions in the innate immune responses of pearl oyster.

2. Materials and methods

2.1. Pearl oyster and immune challenge

Pearl oyster *P. fucata* (body weight 18.2–22.5 g) was obtained from pearl oyster culture base of South China Sea Fisheries Research Institute in Xincun Village, Hainan Province, China and maintained at 25–27 °C in tanks with recirculating seawater for 1 week before experiment. The pearl oyster was fed twice daily on *Tetraselmis suecica* and *Isochrysis galbana* in the whole experiment process. Pearl oysters were injected into the adductor muscle with 100 μl of Phosphate Buffered Saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) as control group. The bacterial challenge group was performed by injecting with 100 μl of *Vibrio alginolyticus* resuspended in PBS to OD_{600} = 0.4 (1 OD = 5×10^8 bacteria ml^{-1}) into the adductor muscles of each pearl oyster. At each time point (0, 2, 4, 8, 12, 24 and 36), digestive gland was collected from control group and bacterial challenge group and stored in liquid nitrogen until used. For tissue distribution analysis, unchallenged pearl oyster's digestive gland, gonad, haemocytes, gills, mantle, adduct muscle and intestine were collected as unchallenged group and stored in liquid nitrogen until used. The same tissues were also collected and stored from bacterial challenge group at 8 h post-injection. Pearl oysters of each group were divided into three replicates with equal amounts and fed in three tanks. Five pearl oysters were randomly sampled from each group at each time point, and mixed corresponding tissues with equal amounts as one sample.

2.2. cDNA library construction and EST analysis

A cDNA library was constructed from the whole body of a pearl oyster challenged by *V. alginolyticus*, using the ZAP-cDNA synthesis kit and ZAP-cDNA GigapackIII Gold cloning kit (Stratagene). Random sequencing of the library using T3 primer yielded 6741 successful sequencing reactions. BLAST analysis of all expressed sequence tag (EST) sequences revealed that an EST of 515 bp (pmpca0_005082) was similar to the galectins of eastern oyster *C. virginica* (DQ779197) and bay scollap *A. irradians* (FJ469998). This EST was selected to further clone the PoGal.

2.3. Cloning the full-length cDNA of PoGal

Based on the identified EST sequence, two gene-specific primers PoGal-F and PoGal-R (Table 1) were designed to amplify the full-length PoGal cDNA by rapid amplification of cDNA ends (RACE) technique. To obtain 5′-end of the PoGal cDNA, PCR reaction was performed in a T-1 Thermocycler (Biometra) with T3 and PoGal-R primers (Table 1) in a 25 μl of reaction volume, containing 2.5 μl of 10× PCR buffer (600 mM Tris–HCl pH 8.3, 250 mM KCl, 1% Triton X100, 100 mM β-mercaptoenthanol), 1.5 μl of $MgCl_2$ (25 mmol L^{-1}), 2.0 μl of dNTP (2.5 mmol L^{-1}), 1 μl of each primer (10 μmol L^{-1}), 15.8 μl of double-distilled water, 0.2 μl (1.0 U) of Ex Taq (TaKaRa) and 1 μl of 100-fold diluted cDNA library as template. The cycle condition was one initial denaturation cycle of 94 °C for 2 min, then 35 PCR cycles of 94 °C for 20 s, 60 °C for 30 s, 72 °C for 1 min, and a final extension step at 72 °C for 5 min. PCR amplification of 3′-end of the PoGal was carried out with T7 and PoGal-F primers (Table 1), the PCR cycling conditions were similar to the previously described. The PCR products were separated by agarose gel (1.2%) electrophoresis, and the bands were excised and purified using a DNA Gel Extraction Kit (TaKaRa). Finally, the purified DNA fragments were cloned into the pMD18-T vector (TaKaRa) and sequenced.

Table 1
Primers used in the present study.

Name	Sequence (5′–3′)	Application
PoGal-F	CCATCAGGATTGGAGAAAGGAG	For RACE PCR
PoGal-R	GAAAGGACTGAAGGGGAAATCT	
T3	AATTAACCCTCACTAAAGGG	
T7	GTAATACGACTCACTATAGGGC	
PoGal-QF	AGATTTCCCCTTCAGTCCTTTC	For Real-time RT-PCR
PoGal-QR	TGAAGAAATTGCATTCATGGAC	
β-Actin-F	GCCGAAAGAGAAATCGTCAG	
β-Actin-R	TGGCTGGAATAGGGATTCTG	
M13–47	CGCCAGGGTTTTCCCAGTCACGAC	For sequencing
RV-M	GAGCGGATAACAATTTCACACAGG	
Oligo dT-adaptor primer	GGCCACGCGTCGACTAGTAT$_{(16)}$	For reverse transcription

2.4. Sequence analysis of PoGal

PoGal amino acid sequence was predicted using DNATool version 6.0 software. The percentage of similarity and identity of the known galectin sequences was calculated using the MatGAT program (Campanella et al., 2003) with default parameters. The protein domain was predicted with the simple modular architecture research tool (SMART) program (Schultz et al., 1998; Letunic et al., 2006). The protein sequence of PoGal was compared to its counterpart sequences currently available in GenBank using BLAST program (Altschul et al., 1997) (http://www.ncbi.nlm.nih.gov). Multiple alignment of PoGal was carried out with ClustalW program (http://www.ebi.ac.uk/clustalw/). The phylogenetic tree was constructed with MEGA program version 4 (Tamura et al., 2007) based on amino acid sequence alignment. The phylogenetic tree was tested for reliability using 1000 bootstrap replications.

2.5. Real-time quantitative RT-PCR analysis of PoGal

The expression pattern of PoGal in digestive gland, gonad, haemocytes, gills, mantle, adductor muscle and intestine from unchallenged group and bacterial challenged group at 8 h post-injection were detected by real-time quantitative RT-PCR. Temporal expression level in digestive gland after bacterial challenge was also detected by real-time quantitative RT-PCR. Total RNA samples were extracted using RNeasy Mini Kit (Qiagen) according to the manufacture's instructions, and treated with DNase I (Qiagen) to remove contaminated DNA. Subsequently, the first-strand cDNA was synthesized based on manufacture's instruction of PrimeScript™ RT reagent Kit (Perfect Real Time) (TaKaRa) using total RNA as template. cDNA mix was diluted to 1:5 and stored at −80 °C for subsequent real-time quantitative RT-PCR. Two PoGal gene-specific primers, PoGal-QF and PoGal-QR (Table 1), were designed to amplify a product of 136 bp. The β-actin gene was used as an internal control to verify the real-time quantitative RT-PCR reaction and adjust the cDNA templates. Two β-actin gene-specific primers, β-actin-F and β-actin-R (Table 1), were designed to amplify a fragment of 183 bp.

Real-time quantitative RT-PCR was performed in a total volume of 20 μl containing 10 μl of 2× SYBR Green Real-time PCR Master