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Cloning, genomic structure, and expression analysis of peroxiredoxin V from bay scallop *Argopecten irradians*

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ABSTRACT

Peroxiredoxins (Prxs) constitute a superfamily of antioxidative proteins that play important roles in protecting organisms against damage from reactive oxygen species (ROS). In this study, the peroxiredoxin V gene from *Argopecten irradians* (Ai-PrxV) was isolated and characterized. The full-length Ai-PrxV cDNA consists of 1689 bp with a 567 bp open reading frame (ORF) that encodes 188 amino acids. Three putative polyadenylation consensus signals (AATAAA) were found in the 953 bp long 3′-UTR. The genomic length of the Ai-PrxV gene is 12575 bp, and it contains six exons and five introns. The gene structure is closely related to those of chordates but differs from those of arthropods. The 5′ flanking region, which contains several putative transcription factor binding sites, was also analyzed. Quantitative real-time PCR analysis showed that the highest expression of the Ai-PrxV transcripts occurred in gill tissue. When challenged with the bacteria *Vibrio anguillarum*, the level of Ai-PrxV transcripts in hemocytes of bay scallops was up-regulated and reached the highest point at 15 h post-challenge. These results indicate that Ai-PrxV is a constitutive and inducible protein that plays an important role in the immune response against bacterial infection.

1. Introduction

Originally introduced from the United States in 1982, bay scallop *Argopecten irradians* has become one of the most important cultured bivalves in China [1,2]. However, the sustainable development of the industry is hampered by several factors such as disease outbreaks, environmental deterioration, and climate change. Understanding the immune mechanism of this species is essential to overcoming these problems and promoting bay scallop aquaculture.

Reactive oxygen species (ROS) are produced by cells as by-product of the metabolism of oxygen during normal biophysical process and increase in response to various stress conditions. They can trigger signal transduction pathways and help killing foreign invaders. However, excessive accumulation of these reactive molecules is detrimental to the cell because of the destructive effect on many cellular components, such as nucleic acids and proteins [3–5]. Under normal condition, the intracellular level of ROS is strictly maintained and controlled by peroxiredoxins (Prxs) and other enzymes. Prxs constitute a family of ubiquitously expressed proteins that play a protective antioxidant role by scavenging H_2O_2 and alkyl hydroperoxides [6,7]. In addition to maintaining the balance of ROS, Prxs also participate in various biological functions, e.g., cell apoptosis, proliferation, differentiation, intracellular signaling, and modulation of gene expression [8–15].

Prxs were first found in the cytosol of yeast (*Sacchromyces cerevisae*) (named TpxI) [16]. Since then, more and more Prx family members have been identified in plants and animals, including invertebrates and vertebrates. Based on the number of cysteine (Cys) residues needed for catalytic activity and the type of disulfide bond formed, the six Prxs from mammals are divided into three categories: 2-Cys (PrxI–IV), atypical 2-Cys (PrxV), and 1-Cys (PrxVI) [17,18]. This classification has been extended to all Prxs. PrxV, first isolated from mammals [19], is an indispensable part in an integrated cellular antioxidant defense network which prevents ROS-mediated damage and ensures that cells respond appropriately to increased levels of oxidative stress through H_2O_2-mediated signaling pathways. Like other Prxs, PrxV is widely expressed in tissues. However, it is localized intracellularly to the cytosol, mitochondria, and peroxisomes [20].

Among the antioxidant enzymes that protect organisms from excessive ROS, the Prx family has received an increasing amount of attention during the past decade. Although PrxVs from *Chlamys farreri* (GenBank accession no: EF634307.1) and *Laternula elliptica* (GenBank accession no: EU734750.1) have been reported, little is

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known about the genomic features of the PrxV gene in mollusks, as well as the role of PrxV in the molluscan immune response against infections. To bridge the gap, our study aims to: (1) determine the nucleotide sequence of *A. irradians* PrxV (Ai-PrxV) cDNA and compare its deduced amino acid sequence with those of other known PrxVs; (2) clone the genomic DNA of Ai-PrxV and characterize the genomic sequence and the 5′ flanking region; (3) examine the expression of Ai-PrxV in various tissues; and (4) investigate the temporal pattern of Ai-PrxV expression in response to an immune challenge via *Vibrio anguillarum* injection.

2. Materials and methods

2.1. Bay scallops

Bay scallops averaging 62 mm in shell length were collected from an aquaculture farm in Qingdao, China. All scallops were acclimatized at 18 °C in aerated seawater for two weeks before being subjected to experimental manipulation. Scallops were fed with 0.5% *Isochrysis galbana* twice per day. Seawater was refreshed every day.

2.2. V. anguillarum challenge and tissue collection for gene expression study

V. anguillarum was cultured in Luria–Bertani broth medium at 28 °C to mid-logarithmic phase then resuspended in PBS (pH 7.2). Bay scallops were randomly divided into challenge group and control group (30 scallops each). Scallops in the challenge group were injected with 50 μl live *V. anguillarum* (2×10^8 CFU ml^{-1}, strain no. MVM425) into the adductor muscle; while scallops in the control group were injected with PBS instead. Four individuals from each group were randomly sampled at 0, 5, 10, 15, 23, 30 h and 48 h after injection. One milliliter of hemolymph was drawn from each animal by using a fresh needle and syringe, and then immediately centrifuged at 3500 g for 3 min to collect hemocytes. Gill, adductor muscle, gonad, hepatopancreas, and hemocytes samples were taken from three healthy scallops under natural conditions (no injection) to study the tissue-specific mRNA expression of Ai-PrxV. Samples were stored at −80 °C until use.

2.3. RNA extraction

Total RNA was isolated from tissues using TRIzol Reagent (Invitrogen, USA). The quality of RNA was assessed by electrophoresis on 1.2% agarose gel. RQ1 RNase-Free DNase (Promega, USA) was used to eliminate DNA contamination. The first strand of cDNA was synthesized with CDS Primer A (Table 1) using M-MLV reverse transcriptase (Promega, USA) according to the manufacturer's recommendations.

2.4. Cloning the full-length cDNA of Ai-PrxV

The PrxV protein sequences from different species were downloaded from GenBank and aligned using the ClustalW multiple sequence alignment program. Two pairs of degenerate primers (PV dp F1 and PV dp R1; PV dp F2 and PV dp R2, Table 1) were designed based on the highly conserved amino acids. The fragment was amplified in a 25 μl reaction volume containing 2.5 μl 10 × PCR buffer (Mg^{2+} plus), 2 μl dNTP mixture (2.5 mM each), 1 μl forward primer (10 μM), 1 μl reverse primer (10 μM), 18.3 μl dd H_2O, 0.2 μl Taq polymerase (5 U/μl) (TaKaRa, Japan), and 1 μl template cDNA. Two rounds of PCR were performed using a My Cycler System (BioRad, USA) with the following touchdown program: 10 cycles at 94 °C for 30 s, 60 °C (−1.3 °C per cycle) for 40 s, and 72 °C for 50 s, then 25 cycles at 94 °C for 30 s, 47 °C for 40 s, and 72 °C for 50 s followed by an extension at 72 °C for 10 min. The PCR product was cloned into pMD18-T vector (TaKaRa, Japan) and subjected to sequence analysis.

Based on the partial sequence known, 3′ end of Ai-PrxV was obtained by rapid amplification of cDNA ends (RACE) using the SMART RACE cDNA amplification protocol. Briefly, the first-strand cDNA was synthesized with CDS Primer A as described above. The first round PCR was performed with the specific primer PV F1 and the universal primer mixture UPM. The second round PCR was performed with the specific primer PV F2 and the nested universal primer NUP (Table 1). The PCR was conducted using the following program: 35 cycles of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 1 min followed by an extension at 72 °C for 10 min.

5′ end of Ai-PrxV was obtained using the commercial 5′-Full RACE Kit (TaKaRa, Japan) according to the manufacturer's instructions. PV R1 and 5′ outer primer were used for the first round PCR, and PV R2

Table 1
Sequences of primers for Ai-PrxV cDNA cloning and qRT-PCR.

Primer	Sequence 5′–3′	Application
cDNA		
PV dp F1	CGGCGTGCCCGGNGCNTTYAC	Degenerate primer, 1st PCR
PV dp R1	CAGCCAGASAACAAGTCAGTCCNGTNCCRTC	Degenerate primer, 1st PCR
PV dp F2	CCCCGGATGYTCCAARACNCAYYT	Degenerate primer, 2nd PCR
PV dp R2	AACAAGTCAGTCCTGTTCCRTCNGGYTCNAC	Degenerate primer, 2nd PCR
CDS primer A	AAGCAGTGGTATCAACGCAGAGTAC(T)30V N	1st strand of cDNA synthesis primer
PV F1	GGAGCGGCTGGCAAGGTCAGAATGTTA	3′ RACE 1st PCR
PV F2	TGGTTGTGAATGACGGGAAGATTGAGA	3′ RACE 2nd PCR
PV R1	CTTCTCAATCTTCCCGTCATTCACAACC	5′ RACE 1st PCR
PV R2	ACCTTGCCAGCCGCTCCTTGATTTTC	5′ RACE 2nd PCR
UPML	CTAATACGACTCACTATAGGGCAAGCAGTGG TATCAACGCAGAGT	Universal primer, 3′ RACE 1st PCR
UPMS	CTAATACGACTCACTATAGGGC	Universal primer, 3′ RACE 1st PCR
NUP	AAGCAGTGGTATCAACGCAGAGT	Universal primer, 3′ RACE 2nd PCR
5′ Outer primer	CATGGCTACATGCTGACAGCCTA	Universal primer, 5′ RACE 1st PCR
5′ Inner primer	CGCGGATCCACAGCCTACTGATGATCAGTCGATG	Universal primer, 5′ RACE 2nd PCR
qRT-PCR		
A rt F	CCCTCTATGCCTCTGGTCGT	β-Actin primer
A rt R	TTCTCTCTCGGCTGTGGTTG	β-Actin primer
PV rt F	AATCAAGGAGCGGCTGGCA	Ai-PrxV primer
PV rt R	TCAACTTCTCAATCTTCCCGTCAT	Ai-PrxV primer