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ScienceDirect
Developmental and Comparative Immunology 31 (2007) 548–558
Developmental & Comparative Immunology
www.elsevier.com/locate/devcompimm

Molecular cloning and mRNA expression of peptidoglycan recognition protein (PGRP) gene in bay scallop (*Argopecten irradians*, Lamarck 1819)

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Received 13 May 2006; received in revised form 29 August 2006; accepted 8 September 2006
Available online 4 October 2006

Abstract

Peptidoglycan recognition proteins (PGRPs) are a type of pattern recognition molecules (PRM) that recognize the unique cell wall component peptidoglycan (PGN) of bacteria and are involved in innate immunity. The first bivalve PGRP cDNA sequence was cloned from bay scallop *Argopecten irradians* by expressed sequence tag (EST) and PCR technique. The full-length cDNA of bay scallop PGRP (designated AiPGRP*)* gene contained 1018 bp with a 615-bp open reading frame that encoded a polypeptide of 205 amino acids. The predicted amino acid sequence of AiPGRP shared high identity with PGRP in other organisms, such as PGRP precursor in *Trichoplusia ni* and PGRP SC2 in *Drosophila melanogaster*. A quantitative reverse transcriptase Real-Time PCR (qRT-PCR) assay was developed to assess the mRNA expression of AiPGRP in different tissues and the temporal expression of AiPGRP in the mixed primary cultured hemocytes challenged by microbial components lipopolyssacharide (LPS) from *Escherichia coli* and PGN from *Micrococcus luteus*. Higher-level mRNA expression of AiPGRP was detected in the tissues of hemocytes, gonad and kidney. The expression of AiPGRP in the mixed primary cultured hemocytes was up regulated after stimulated by PGN, while LPS from *E. coli* did not induce AiPGRP expression. The results indicated that AiPGRP was a constitutive and inducible expressed protein that was mainly induced by PGN and could be involved in scallop immune response against Gram-positive bacteria infection.

Keywords: *Argopecten irradians*; Peptidoglycan recognition protein; Expressed sequence tag; cDNA cloning; mRNA expression

1. Introduction

The innate immune system is a phylogenetically ancient mechanism of host defense found in essentially every multicellular organism. In invertebrates, innate immunity is the only mechanism of defense, which includes a cellular response mediated by hemocytes and a humoral immune response that employs constitutive and inducible extracellular molecules [1,2].

The innate immune system develops effective strategies to discriminate non-self invading pathogens from self-tissues. The major targets of innate immune recognition are pathogen-associated

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doi:10.1016/j.dci.2006.09.001

molecular patterns (PAMPs) of microorganisms that are structurally distinct from anything produced by the hosts. PAMPs include parts of the microorganism cell envelope, such as lipopolyssacharide (LPS), peptidoglycan (PGN) and related molecules. The PAMPs are recognized by a limited number of germ-line-encoded receptors, termed pattern-recognition receptors (PRRs). In invertebrates, an increasing number of PRRs have been found, such as lectin [3–5], hemolin[6], lipopolysaccharide-binding protein (LBP) [7], Toll-like receptor(TLR) [8], peptidoglycan recognition protein (PGRP) [9,10] and β-1, 3 glucan recognition protein (ßGRP)[11,12].

PGN is an essential cell wall component in virtually all bacteria, and it is an excellent target for recognition by the eukaryotic innate immune system. There are two types of PGN in nature, L-lysine type (Lys-type) PGN from Gram-positive bacteria and meso-diaminopimelic acid-type (Dap-type) PGN from Gram-negative bacteria [13,14]. PGN can induce strong anti-bacterial responses in insects [15,16] and activates monocytes, macrophages, and B lymphocytes in mammals [17–20].

PGRP, a kind of PRR recognizing and binding PGN, was first identified as a 19-kDa protein from haemolymph and cuticle of silkworm *Bombyx mori* [21]. Subsequently, PGRP genes have been identified in many organisms, especially insects and mammals, such as *Trichoplusia ni* [22], *Anopheles gambiae* [23], *Drosophila melanogaster* [24], *Homo sapiens* [25], *Mus musculus* [22,26] and *Bos taurus* [27]. Only a few PGRP genes or gene fragments have been cloned from marine animals, including squid *Euprymna scolopes* [28], starfish *Asterias rubens* [GenBank No. ABB04460], and a PGRP homologous fragment from *Argopecten irradians* [GenBank No. CK484227].

Based on the predicted structures, PGRPs have been divided into three classes: small extracellular PGRPs (PGRP-S) of 20–25 kDa, intermediate PGRPs (PGRP-I) of 40–45 kDa which have two predicted transmembrane domains, and long PGRPs (PGRP-L) of up to 90 kDa which have long transcripts and are either intracellular or membrane-spanning proteins.

The knowledge about the functions of different PGRPs is mainly from insects and mammals. The previous studies validated the distinct specificities of PGRPs to bind PGNs from different microbes. In fruit fly, PGRP-SA recognizes Lys-type PGN, which triggers the Toll receptor pathway that mediates response to fungi and Gram-positive bacteria [29,30]; while PGRP-LC and PGRP-LE interact with Dap-type PGN and activate the Imd/relish pathway which constitutes essential components to recognize Gram-negative bacteria [31–37]. Meanwhile, PGRPs have also been found to have other functions. For example, Human and mouse PGRP-L share the same function with several *Drosophila* PGRPs [38,39] to hydrolyze the amide bond of PGNs and probably act as scavengers [40,41]. Mouse PGRP-S can inhibit the growth of certain Gram-positive bacteria and participates in the intracellular killing of bacteria in neutrophils [42]. Accordingly, the mice with PGRP-S gene knockout exhibited increased susceptibility to intraperitoneal infections with Gram-positive bacteria [43]. Bovine PGRP-S is bacteriostatic or bacteriocidal for both Gram-positive and Gram-negative bacteria [27]. In addition, mouse PGRP-S has been reported to form a potent cytotoxic complex with heat shock protein 70 (Hsp70) that induces apoptotic death in various tumor lines [44,45].

To our knowledge, molecular features and functional studies in invertebrate PGRPs still remain deficient. More information about PGRP can help us to better understand the interaction between the invading microorganisms and the host animals. As the second largest group of the invertebrate phylum, mollusks attract special attentions because of their economic importance. One of the marine mollusks, bay scallop *A. irradians* is being cultured extensively in China. The industry of bay scallop culture has expanded rapidly since it was introduced from America in 1982. After flourishing for several years, the bay scallop culture in China has been suffering from the problem of mortality. Understanding the immune defense mechanisms of scallop may help to develop strategies for the control of scallop diseases and help the long-term sustainability of scallop farming. The main objectives of the present study are (1) to clone the first bivalve PGRP gene from scallop *A. irradians*; (2) to investigate the expression of AiPGRP in different tissues and its temporal expression in the mixed primary cultured hemocytes challenged by LPS and PGN; and (3) to provide information to understand the roles of AiPGRP in scallops' immune response.

2. Materials and methods

2.1. cDNA library construction and EST analysis

A cDNA library was constructed from the whole body of a bay scallop, using the ZAP-cDNA