Available online at www.sciencedirect.com
SCIENCE DIRECT
Molecular Immunology 43 (2006) 1633–1644
Molecular Immunology
www.elsevier.com/locate/molimm

Identification and molecular characterization of a peritrophin-like protein from fleshy prawn (*Fenneropenaeus chinensis*)☆

Xin-Jun Du [a], Jin-Xing Wang [a,*], Ning Liu [a], Xiao-Fan Zhao [a], Fu-Hua Li [b], Jian-Hai Xiang [b]

[a] *School of Life Sciences, Shandong University, Jinan 250100, Shandong, China*
[b] *Institute of Oceanology, The Chinese Academy of Sciences, Qingdao 266071, Shandong, China*

Received 23 August 2005; accepted 24 September 2005
Available online 3 November 2005

Abstract

Peritrophin, one of the components of the peritrophic matrix, was first isolated from the intestine of insects. It is thought to protect insects from invasion of microorganisms and to stimulate digestion of food. Peritrophin-like proteins have also been found in crustaceans, as a component of the egg layer. In this study, one fragment of the peritrophin-like gene was obtained from fleshy prawn (Chinese shrimp) (*Fenneropenaeus chinensis*) by panning the T7 phage display library constructed with the shrimp hemocyte cDNA. The total sequence of the peritrophin cDNA was cloned by modified SMART cDNA and LD-PCR methods. The full cDNA is 1048 bp and the deduced protein is composed of 274 amino acids, including 21 amino acid signal peptide, and four peritrophin A domains and the latter three forming three chitin-binding domains. Similarity analysis results showed that the peritrophin-like protein from *F. chinensis* has significant similarities with peritrophin-like and cortical rod proteins from other shrimp. It was inducing expression in hemocytes, heart, stomach, gut, and gills of the infected shrimp, and constitutive expression in the ovaries. No expression signal was detected in the hepatopancreas of either infected or noninfected shrimp. The recombinant peritrophin-like protein has the activity of binding Gram-negative bacteria and strong binding activity to chitin. Therefore, the bacteria and chitin binding activities of the peritrophin-like protein suggest that it may plays a role in immune defense and other physiological resposes.

Keywords: Peritrophin-like gene; Expression pattern; Fleshy prawn; *Fenneropenaeus chinensis*; Chitin binding activity; Immune defense

1. Introduction

A noncellular semipermeable membrane called the peritrophic matrix (PM) lines the midgut of insects. The presence of this matrix has been noted for centuries, yet its function has not been well defined (Tellam et al., 1999). It is believed that the PM facilitates digestion and forms a protective barrier to prevent the invasion of bacteria, viruses and parasites (Lehane, 1997). Two types of PMs have been defined, based on the location of synthesis in the organism (Peters, 1992). Type 1 PM is synthesized by all midgut epithelial cells and can be constitutively produced. It forms a bag-like structure that can hold ingested nutrients. Type 2 PM is constitutively synthesized by cardia, a small organ located in the anterior region of the midgut, and forms open-ended sleeve-like structures (Tellam et al., 1999). PMs are composed of glycoproteins, proteoglycans, proteins and chitin. More is known about type 2 PM than type 1 PM, because type 2 is easier to obtain (Tellam et al., 1999).

At our current level of knowledge, PM proteins were divided into four classes based on their differential extractions from the matrix using solubilising conditions of increasing severity (Tellam, 1996, 1999). The first class of proteins can be easily removed using physiological buffers. The second class can be extracted by relatively gentle detergents. The third class of proteins is thought to be integral to the PM and can only be removed by strong denaturants, such as urea, guanidine hydrochloride or sodium dodecyl sulphate (SDS). The residue that remains after stringent extraction includes the fourth class of proteins, but little is known about these proteins.

The third class of proteins, the peritrophins, is the most extensively studied of the four classes (Elvin et al., 1996). Peritrophins have been found and sequenced in several invertebrates, such as Australian sheep blowfly, *Lucilia cuprina* (Elvin et al., 1996; Schorderet et al., 1998; Tellam et al., 2000,

☆ The sequence reported in this paper has been deposited in the GenBank database (accession no: DQ091253).

* Corresponding author. Tel.: +86 531 88384620; fax: +86 531 88565610/364620.
E-mail address: jxwang@sdu.edu.cn (J.-X. Wang).

0161-5890/$ – see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.molimm.2005.09.018

2003), African malaria mosquito, *Anopheles gambiae* (Shen and Jacobs-Lorena, 1998), Old World screwworm fly, *Chrysomya bezziana* (Tellam, 1996) and cat flea (*Ctenocephalides felis*) (Gaines et al., 2003). Every identified peritrophin has a signal sequence and one of three kinds of peritrophin domains (peritrophin-A, B or C).

Recent research has demonstrated that peritrophins also exist in crustaceans. Khayat et al. found that two peritrophin-like cDNAs were highly expressed during oogenesis in shrimp (*Penaeus semisulcatus*). They had peritrophin-A-like domains marked with six-cysteine residues, and were named shrimp ovarian peritrophins (SOPs). SOPs are a major proteins in the ovaries and are extruded from the egg cortical crypts to form a protective layer around eggs immediately after spawning (Avarre et al., 2001; Khayat et al., 2001). Kim et al. purified two cortical rod proteins and cloned the two full-length cDNAs based on the N-terminal sequences of the proteins from *Marsupenaeus japonicus*. The cDNAs resembled shrimp ovarian peritrophin (SOP) in *Penaeus semisulcatus*, which was homologous to insect peritrophin. They determined that both molecules are components of the cortical rods, forming a jelly layer after fertilization.

The fragment of peritrophin-like gene used in this study was obtained by panning the T7 phage display library, which was constructed from hemocyte cDNA of fleshy prawn (originally denominated Chinese shrimp), *Fenneropenaeus chinensis* using *E. coli*. Based on this sequence, complete peritrophin-like cDNA was acquired using modified SMART (Switching Mechanism At 5′ end of RNA Template) cDNA and the LD-PCR (long distance PCR) method, and the expression profile of peritrophin transcript was then analyzed. The peritrophin-like gene from *F. chinensis* was denominated Fc-peritrophin or FCP in short. The mature FCP was expressed in *Escherichia coli*, and the characteristic of the protein was studied.

2. Materials and methods

2.1. Immunity challenge of shrimp and hemocytes collection

F. chinensis shrimp (about 10–20 g) were obtained from a shrimp farm in Qingdao, Shandong province, PR China, and cultured in the laboratory in 500 L tanks (at 25 °C) filled with air-pumped circulating sea water. A cocktail (3×10^7 cells/per animal) of microorganisms, including *Staphylococcus aureus* and *Vibrio anguillarum*, was injected into the abdominal segment of the shrimp. Haemolymph was taken from the ventral sinus 12 h after injection and immediately centrifuged at $800 \times g$ for 5 min (4 °C) to isolate the hemocytes from the haemolymph. Using the same method, hemocytes were collected from unchallenged shrimp from the laboratory tanks.

2.2. Construction of phage display library of fleshy prawn hemocyte cDNA

The T7 phage display library was constructed following T7 Select System procedures (Novagen, Madison, WI). Briefly, mRNA was extracted from the hemocytes of challenged shrimp using the QuickPrep micro mRNA Purification Kit (Amersham Biosciences). Reverse transcription was used to synthesize the first strand of cDNA using 4 μg mRNA from hemocytes and random primers. Then double stranded cDNA was obtained using DNA polymerase. After extraction with phenol and modification of the ends, *Eco*RI/*Hind*III linkers were added at two ends of the double strand cDNA. *Hind*III and *Eco*RI were used to cleave the double strand cDNA, and then the cDNA was ligated to T7 select vector arms after size fraction. The ligated inserts and vector arms were packed with 25 μl of T7 Packaging Extracts. Finally, the library was amplified after the phage titer was calculated.

2.3. Screening the phage display library by biopanning and sequencing

Formaldehyde-fixed *E. coli* were used to pan the T7 phage display library. The bacteria were washed twice with TBS (50 mM Tris–HCl, 0.5 M NaCl, pH 7.5) before fixing with 3.5% paraformaldehyde. After washing, 100 μl of amplified T7 phage library was added to 100 μl of bacteria ($OD_{600} = 2.0$) then mixed gently and keep at 4 °C overnight. The mixture was centrifuged at $6000 \times g$ for 5 min and the supernatant was discarded. The bacteria bound with phage were washed with TBST (50 mM Tris–HCl, 0.5 M NaCl, 0.02% Tween 20, pH 7.5) three times and then resuspended with 200 μl 1% SDS and kept for 10 min. The bacteria with phage was then centrifuged at $6000 \times g$ for 5 min and the supernatant was removed to a new tube. The eluted phage (10 μl) was added to 1 ml BLT5403 host cells and agitated at 37 °C for 1 h. After centrifuging, 200 μl of supernatant with eluted phage was amplified in an agarose plate with BLT5403 host cells at 37 °C for 3 h. Five milliliters of extraction buffer was added to plates with sufficient plaques and keep at 4 °C overnight. The extracted phage was collected for the second panning. After three rounds of panning, 10 plaques were randomly selected from the plate for PCR amplification and sequencing.

2.4. 5′ and 3′ end cloning of Fc-peritrophin gene

Modified SMART (Switching Mechanism At 5′ end of RNA Template) cDNA and the long distance PCR (LD-PCR) procedures of CLONTECH (Palo Alto, CA) were used to complete the 5′ and 3′ ends of the gene. First strand cDNA was reverse transcribed from 5 μg total RNA with Oligo-anchor R primer and Smart F primer (Table 1) using the First Strand cDNA Synthesis Kit (Sangon, Shanghai). 5′ PCR primer and 3′ anchor R primer were used to produce the LD-PCR library. The 3′ end of the gene was amplified using a gene-specific forward primer PTPH F and 3′ anchor R primer. The following procedure was used: one cycle at 94 °C for 2 min; 35 cycles at 94 °C for 30 s; 53 °C for 45 s; 72 °C for 1 min, followed by one cycle at 72 °C for 15 min. 5′ PCR primer and a reverse gene-specific primer PTPH R were used to clone the 5′ end of the gene. PCR procedure is as follows: one cycle at 94 °C for 2 min; 35 cycles at 94 °C for 30 s; 53 °C for 45 s; 72 °C for 45 s, followed by one cycle at 72 °C for 15 min. Sequences of all primers were list in Table 1.