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Hepatopancreatic nuclease of black tiger shrimp *Penaeus monodon* unlikely to be involved in viral triggered apoptosis ☆

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Abstract

Nucleases are phosphodiesterases that hydrolyze DNA and/or RNA. In a search for shrimp nucleases involved in apoptosis, we discovered a nuclease from hepatopancreatic cDNA of the black tiger shrimp *Penaeus monodon*. The full-length nuclease gene was amplified and revealed to contain 1668 bp corresponding to 381 deduced amino acid residues in the mature enzyme. Sequence analysis indicated 83% nucleic acid identity and 89% amino acid identity to a nuclease from the Kuruma shrimp *Penaeus japonicus* (also called *Marsupenaeus japonicus*). Comparative analysis of sequences, conserved motifs and phylogenetic trees indicated that *P. monodon* nuclease (PMN) belonged to the family of DNA/RNA non-specific endonucleases (DRNSN). RT–PCR analysis using primers specific for PMN mRNA with seven different shrimp tissues revealed that expression in normal shrimp was restricted to the hepatopancreas. Semiquantitative RT–PCR analysis of PMN using hepatopancreatic mRNA from normal shrimp and from shrimp challenged with white spot syndrome virus (WSSV) indicated significant up-regulation of PMN in the hepatopancreas ($P < 0.05$) at the early stage of viral infection but a return to baseline levels as gross signs of disease developed. At the same time, expression was always confined to the hepatopancreas and never seen in other tissues, including those reported to be prime targets for WSSV and subject to increased levels of apoptosis after infection. The results suggested that PMN is probably a digestive enzyme that is unlikely to be involved in hallmark DNA digestion associated with apoptosis.

Keywords: Nucleases; *Penaeus monodon*; White spot syndrome virus; Tissue specificity; Apoptosis; Shrimp

1. Introduction

Nucleases hydrolyze nucleic acids (DNA and RNA) by cleaving the phosphodiester bonds between nucleotides. They are grouped based on specificity for the sugar moiety in their polynucleotide targets as deoxyribonucleases

☆ The GenBank accession number for the PMN sequence reported in this paper is ABF69938.

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(DNase), ribonucleases (RNase) and sugar non-specific nucleases (abbreviated as ''nucleases'') that hydrolyze both DNA and RNA [1]. Here, we will refer to the latter specifically as NSN. Many reports have shown that NSN play an important role in biological functions such as the removal of RNA primers from Okazaki fragments [2], embryogenesis and tissue turnover [3,4]. They may also be involved in apoptosis [5–9] for which internucleosomal DNA digestion is considered a biochemical hallmark that distinguishes apoptosis from necrosis [10]. In shrimp, apoptosis has been causally implicated in the death of shrimp from infections by white spot syndrome virus (WSSV) [11,12] and yellow head virus (YHV) [13]. For both viruses, assays of DNA digestion were used as key indicators of apoptosis. Thus, we were interested in isolation and characterization of the shrimp nuclease that might be involved in the process.

Several candidate apoptotic nucleases have been described, some showing specific, optimal pH or bivalent cation requirements or specific tissue expression [14]. However, in many cases, the role of specific nucleases in apoptosis is still unclear [10]. Deoxyribonuclease I (DNase I) from the Kuruma shrimp *Penaeus japonicus* (also called *Marsupenaeus japonicus)* was purified and found to have functional activity similar to that of bovine DNase I, although nucleotide and protein sequences for the two enzymes are quite different [15]. The shrimp nuclease has been newly renamed as an NSN [16].

The DNA-specific nuclease, bovine DNase I (EC 3.1.21.1) is the best studied. It is found mainly in digestive tissues [17] where it acts upon single stranded [18] and double stranded DNA to produce 5′-phosphate nucleotides [19]. There are suggestions that the degradation of DNA during apoptosis is functionally indistinguishable from that which occurs by DNase I [20]. In summary, it is possible that both NSN and DNase I may play a role in DNA digestion associated with apoptosis.

In this study, we focused on the isolation and characterization of a tissue-specific DRNSN gene from the black tiger shrimp (*P. monodon*). In addition, we examined the differential expression of this gene in normal shrimp and shrimp challenged with white spot syndrome virus (WSSV) to see whether there was any indication that it might play a role in apoptosis.

2. Materials and methods

2.1. Black tiger shrimp culture

Black tiger shrimp (*P. monodon*) fresh weight approximately 20 g were obtained from a shrimp farm in Pathum Thani province (Thailand) and acclimated for 3 days in a 50 × 30 × 30 cm styrofoam box containing 15 ppt artificial sea water.

2.2. Total RNA isolation and reverse transcriptase–PCR (RT–PCR)

The hepatopancreas was dissected within 5 min after the shrimp were immobilized in ice water. Total RNA was extracted by using Trizol reagent (Invitrogen) according to the manufacturer's protocol. *P. japonicus* was used as the model to design primers to amplify the nuclease gene using the *P. monodon* RNA as a template. RT–PCR was done using a SuperScript™ III One-Step RT–PCR kit with Platinum® Taq. The protocol comprised one initial step of 50 °C for 30 min and 94 °C for 2 min followed by 35 cycles of 94 °C for 15 s, 45 °C for 30 s, 72 °C for 1.5 min and final extension at 72 °C for 7 min. Primers were DNSF (5′-TGT GGA ATA AGG ACA CCG ACT T-3′) and DNSR (5′-ATG TCC TTG GCC CGC ATG TTG-3′), synthesized by Proligo Singapore Ple Ltd.

2.3. Rapid amplification of cDNA ends (RACE)

A total of 5 μg of isolated RNA was used and reverse transcribed to cDNA using a GeneRacer™ Kit (Invitrogen). Both 5′ and 3′ ends of cDNA were amplified by PCR using the Advantage™ 2 PCR Enzyme Systems (BD Biosciences), according to the manufacturer's recommendation. The specific pair of primers (Table 1) used was synthesized by Proligo Singapore Ple Ltd. Amplification conditions for both 3′ and 5′ RACE were performed according to the GeneRacer Kit protocol.