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Penaeus monodon caspase is targeted by a white spot syndrome virus anti-apoptosis protein ☆

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Abstract
Caspases play a central and evolutionarily conserved role in mediating and executing apoptosis. Here, we report the cloning and characterization of a caspase from *Penaeus monodon*, Pm caspase. The full-length *Pm caspase* cDNA is 1386 bp, encoding a polypeptide of 304 amino acids with a calculated molecular mass of 34.3 kDa. BLASTP analysis against the NCBI nr database showed that Pm caspase is similar to insect effector caspases. RT-PCR analysis showed that Pm caspase mRNA is expressed in all examined tissues. When Pm caspase was overexpressed in SF-9 cells, the cells showed apoptotic morphological features, including the formation of apoptotic bodies and DNA ladders. The caspase-3 activity of Pm caspase was determined using the recombinant protein purified from *Escherichia coli*. Both RT-PCR and qRT-PCR analyses showed that the RNA levels of *Pm caspase* and *P. monodon inhibitor of apoptosis protein* (*PmIAP*) remained unchanged after white spot syndrome virus (WSSV) infection. We also used Pm caspase to show that WSSV449, an anti-apoptosis protein encoded by WSSV, is a direct caspase inhibitor.

1. Introduction

Apoptosis, a cell suicide program that is used to remove surplus or damaged cells, plays important roles during embryonic development and in the adult [1]. Since the first introduction of the concept of apoptosis in 1972 by Kerr et al. [2], many studies have shown that the underlying mechanisms and molecules involved are highly conserved from mammals to nematodes [3]. Among these molecules, there is a large family of cysteine proteases that play a central and evolutionarily conserved role in mediating and executing apoptosis. These cysteine proteases specifically

Abbreviations: IAP, inhibitor of apoptosis protein; WSSV, white spot syndrome virus; UTR, untranslated region.

☆GenBank accession number of *Penaeus monodon* caspase: EF114674.

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cleave proteins at sites that follow aspartate (Asp) residues, and they are therefore called caspases.

Caspases are the principal executioners of apoptosis, and due to their destructive nature they need to be tightly controlled. They are produced in cells as inactive zymogens, and must undergo proteolytic cleavages at internal Asp sites to become activated. The inactive caspase zymogen consists of an amino-terminal prodomain, a large subunit, and a small carboxyl-terminal subunit. After proteolytic cleavage to separate the large and small subunits, the active caspase forms a heterodimer consisting of two large and two small subunits [4].

Caspases are classified as either initiator or effector caspases. The initiators have a long prodomain (>90 amino acids) that contains specific protein–protein interaction motifs that are necessary for their activation, whereas the effectors usually have a short prodomain with only 20–30 residues. After receiving apoptotic signals, the initiator caspases are auto-activated through the assembly of a multi-component complex made up of the initiator caspases themselves and other proteins [5]. The activated initiators in turn activate the effector caspases by cleaving them at specific internal Asp residues. The activated effector caspases then cleave an array of cellular targets and destroy the cellular architecture, which ultimately results in the demise of the cells [6].

Recent studies in animal host–virus responses have shown that apoptosis plays important roles in host defense mechanisms against viral infection [7,8]. The apoptosis phenomenon has been well documented in shrimps infected with several viral pathogens [9–11]. The above studies showed that the characteristics of viral-induced apoptosis in shrimp are similar to those found in other animals, and that virus-induced pathogenesis in shrimps can be partly attributed to the apoptosis triggered by the virus. However, so far, only two apoptotic molecules have been identified in shrimp: a caspase gene (*Fm caspase*) has been cloned from the shrimp *Fenneropenaeus merguiensis* [12] and an inhibitor of apoptosis protein (IAP) gene has also recently been cloned from *Penaeus monodon* in our lab [13]. The shrimp virus white spot syndrome virus (WSSV) has also been shown to encode a novel anti-apoptosis protein WSSV449 [14]. To further understand the apoptotic interplay between WSSV and the shrimp host, we therefore cloned and characterized a caspase from *P. monodon*. We further demonstrated that the apoptotic responses induced by Pm caspase in insect cells were completely blocked by WSSV449, and only partially blocked by PmIAP.

2. Materials and methods

2.1. Cloning of Pm caspase

A partial *P. monodon caspase* cDNA fragment was first cloned using degenerate PCR. Briefly, mRNA was isolated from *P. monodon* hepatopancreas using the QuickPrep Micro mRNA Purification Kit (GE Healthcare) according to Leu et al. [13], and 200 ng mRNAs were primed with oligo-dT-anchor primer (Roche) and reverse-transcribed with SuperScript II (Invitrogen, USA) at 42 °C for 50 min. Following Pei et al. [15], degenerate primers, 5′-CA(A/G)GC(A/C/G/T)TG(T/C)CA(A/G)GG(A/C/G/T)GA-3′ and 5′-TGCAT(A/G)(A/T)ACCA(A/C/G/T)GA (A/C/G/T)CC-3′, were designed based on the consensus amino acid sequence of the members of the insect caspase family. PCR was performed for 40 cycles of 95 °C for 30 s, 45 °C for 30 s, and 72 °C for 1 min. Amplified fragments were cloned into the pGEM-T easy vector (Promega) and then sequenced.

5′ RACE was performed using the FirstChoice® RLM-RACE kit from Ambion, Inc. according to the manufacturer's protocol. Briefly, about 200 ng poly(A) RNA was used, and after being reverse-transcribed with random decamers, the resulting cDNA was then subjected to nested PCR using two *Pm caspase* antisense primers, 5′-AGGTATAGTGGACCAG-CACTCCAG-3′ and 5′-CGGCGTCGATCTCGTCCTGCTTG-3′, and the two sense primers provided by the kit. For 3′ RACE, the cDNA synthesized from the poly(A) RNA using the oligo(dT)-anchor primer (Roche Molecular Biochemicals) was subjected to PCR using the *Pm caspase* sense primer, 5′-GCACGACCCTCAAGCAGGACGAG-3′, and the anchor primer (Roche Molecular Biochemicals). The PCR products of both 5′ and 3′ RACE were cloned into the pGEM-T easy vector (Promega) and sequenced.

2.2. Sequence analysis of Pm caspase

The deduced amino acid sequence of Pm caspase was analyzed with BLASTP against the NCBI non-redundant (nr) database and with ScanProsite. For multiple sequence alignment and phylogenetic relationship analysis, the following insect, shrimp and vertebrate effector caspases were used: *Drosophila melanogaster* Dcp-1 (NP_476974.1) and Drice (NP_524551.2), *Drosophila pseudoobscura* caspase (Dscasp; XP_001361093), *Bombyx mori* caspase-1 (Bmcasp; AAN86250), *Trichoplusia ni* caspase-1 (Tncasp; AAO17788), *Helicoverpa armigera* caspase-1 (Hacasp; ABS18284), *Spodoptera littoralis* caspase-1 (Slcasp; AAO16241) *Spodoptera frugiperda* caspase-1 (Sfcasp; AAC47442.1), *F. merguiensis* caspase (Fmcasp; AY839873), *Takifugu rubripes* caspase-3 (Trcasp3; AAM43816), *Danio rerio* caspase-3 (Drcasp3; NP_571952), *Gallus gallus* caspase-3 (Gacasp3; NP_990056), *Bos taurus* caspase-3 (Btcasp3; NP_001071308), *Xenopus tropicalis* caspase-7 (Xecasp7; CAJ82745), *Mus musculus* caspase-7 (Mmcasp7; NP_031637), *Rattus norvegicus* caspase-7 (Rncasp7; NP_071596), and human caspase-3 (Hcasp3; CAC88866). In this analysis, *Caenorhabditis elegans* caspase Ced3 (AAG42045) was used as an outgroup. Only the region containing the highly conserved catalytic p20/p10 domains was used for alignment. The multiple sequence alignment was performed using the ClustalX program ver 1.83 with default parameters, and further edited manually for maximal homology. Phylogenetic analysis based on the above alignment was conducted by the Neighbor-Joining and Minimum Evolution methods of the MEGA program version 3.1 [16]. The confidence of the results was tested by performing 1000 bootstrap re-samplings of the data.

2.3. Tissue distribution of Pm caspase mRNA

Total RNAs were extracted from various tissues using TRIzol reagent (Invitrogen, USA). After treating with DNase I, total RNAs were primed with oligo-dT-anchor primer (Roche) and