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SCIENCE DIRECT

Aquaculture 252 (2006) 114–120

Aquaculture

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Molecular cloning and expression of caspase from white shrimp *Penaeus merguiensis*

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Received 7 February 2005; received in revised form 9 July 2005; accepted 25 July 2005

Abstract

Apoptosis is a fundamental cellular process that plays a critical role in normal development and tissue homeostasis, eliminating from an organism unwanted cells, including damaged and virus-infected cells. In order to investigate the significance of apoptosis in response to viral infections in crustacean, we have cloned a caspase gene (*cap-3*) from the white shrimp, *Penaeus merguiensis* infected with white spot syndrome virus (WSSV). The deduced amino acid sequence of the protein named CAP-3 was 320 amino acids in length and contained the highly conserved pentapeptide QACRG recognized as the active site of caspase. CAP-3 showed similarities to the insect caspase, *Spodoptera frugiperda* Sf caspase-1 and the caspases of *Spodoptera littoralis*, *Bombyx mori*, *Drosophila melanogaster* and *Anopheles gambiae*, which in turn belong to the group of human caspase-3, caspase-7 and CED-3. The recombinant CAP-3 protein prepared from *Escherichia coli* had enzymatic activity when assayed using a luminescent substrate specific for the cleavage target site for human caspase-3, implying that CAP-3 functioned as caspase-3. There was a high level of expression of this gene product in moribund shrimp infected with WSSV.

Keywords: Apoptosis; Caspase-3; *Penaeus merguiensis*; White spot syndrome virus (WSSV); Shrimp

1. Introduction

Commercial shrimp farmers have experienced viral disease outbreaks that have caused significant production losses throughout the world (Flegel, 1997). The most virulent causative agent is white spot syndrome virus (WSSV) (Lightner, 1996). The disease was first identified in China and Japan and then spread to other countries with shrimp farming industries (Inouye et al., 1994; Nakano et al., 1994; Takahashi et al., 1994; Wang et al., 1995; Wongteerasupaya et al., 1995; Lo et al., 1996, 1999).

It is well known that crustaceans possess humoral and cellular defenses against microbial pathogens such as bacteria and fungi (Lee et al., 2003; Patat et

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doi:10.1016/j.aquaculture.2005.07.024

al., 2004) but their response to viral pathogens is less understood (Flegel, 2001). Flegel and Pasharawipas (1998) presented a theory of viral accommodation that proposed that shrimps were capable of acquiring specific tolerance to viral pathogens by a process involving viral binding and memory. Although the molecular mechanisms involved in viral accommodation are unknown, it was proposed that they are based on membrane binding and signal transduction pathways involving specific memory that leads to suppression of viral-triggered apoptosis and to persistent innocuous infections. Investigations on yellow head virus (Khanobdee et al., 2002) and WSSV (Wongprasert et al., 2003) in *Penaeus monodon* demonstrated that increasing numbers of apoptotic cells occur post infection and may contribute to shrimp mortality. Thus, the nature and control over induced apoptosis in shrimp deserves further attention.

In recent years, much effort has been made to identify the components of the apoptosis pathway that originate from both hosts and their viruses. For example, the anti-apoptotic gene p35 has been described from nuclear polyhedrosis viruses of the alfalfa looper, *Autographa californica* (AcNPV) (Clem et al., 1991) and the silkworm *Bombyx mori* (BmNPV) (Kamita et al., 1993), while inhibitors of apoptosis (IAPs) has been described from granulosis viruses of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (Birnbaum et al., 1994) and the codling moth, *Cydia pomonella* (Miller et al., 2002). Antiapoptotic activity by p35 was attributed to its ability to potently inhibit Sf caspase-1 activity, blocking the host apoptotic response and allowing viral multiplication to occur (Ahmad et al., 1997; Seshagiri and Miller, 1997; Bump et al., 1995; Xue and Horvitz, 1995). An apoptosis suppressor P49, isolated from *Spodoptera littoralis*, is capable of inhibiting effector caspases from both insects and humans (Pei et al., 2002; Zoog et al., 2002). *Drosophila* caspase-1 (DCP-1) is a cysteine protease and has biochemical properties similar to those of *Caenorhabditis elegans* cell death protease CED-3 (Song et al., 1997). CED-3, a nematode caspase that is closely related to mammalian caspase-3 is a central component of the cell death pathway in developing nematodes (Kuida et al., 1996). Taken together, these results show that the apoptotic program is an evolutionarily conserved program throughout the animal kingdom and that it is mediated, in part, by active caspase(s).

Caspases are aspartate-specific cysteine proteases (Alnemri et al., 1996). They are synthesized as proenzymes that can be activated by autoprocessing, by other caspase family members or by other proteases involved in induction of apoptosis. Thus, caspases were an attractive target in our work on the shrimp response to viral pathogens.

By using evolutionarily conserved genetic sequences, we isolated and partially characterized the caspase-3 gene (*cap-3*) from *Penaeus merguiensis*. The enzyme produced by this gene is an important component of the apoptosis cascade, so it will be useful in identifying and studying the function of other components in the cascade. Understanding the mechanisms of programmed cell death in shrimp will not only expand knowledge on the significance of apoptosis in the invertebrate response to viral pathogens but may also lead to identification of pathway components that will be useful in controlling shrimp diseases caused by viruses.

2. Materials and methods

2.1. Isolation of cap-3 cDNA

A partial sequence of *cap-3* was amplified by RT-PCR using primers that were designed from a conserved region of the caspase gene isolated from various species: the forward primer was 5′-ATG TCT ATG TTG GCG GAA CCA AGG-3′ and the reverse primer was 5′-CAC CAT TCA ACT TCA CCT GCT GAC-3′. To obtain the full-length *P. merguiensis* caspase-3 cDNA, 5′-RACE was performed using the RACE system (Invitrogen) with PCR primers as follows: GSP1, 5′-CAG CGC CCC GAG GTC GAT TG-3′; GSP2, 5′-GTC CGT CCA CGA CCG TGT ATG C-3′ and GSP3, 5′-CCC CTG TTC TCC GTC GTG TTG-3′. The primers for 3′-RACE were GSP1, 5′-CCC AGG CTT TCC AGC TTT CAC-3′ and GSP2, 5′-GGG AGT GTC TTC ATC CAC TAC C-3′. All the PCR products were cloned into pGEM-TEasy (Promega Corporation) and sequenced both strands. DNA sequencing was performed by the dideoxy chain termination procedure using the automated sequencer ABI prism 377. Gene database