Prophenoloxidase has a role in innate immunity in penaeid shrimp

FERNAND F. FAGUTAO, HIDEHIRO KONDO, TAKASHI AOKI and IKUO HIRONO

Laboratory of Genome Science, Tokyo University of Marine Science and Technology
Konan 4-5-7, Minato, Tokyo Japan 108-8477

ABSTRACT

Melanization, which is brought about by the activation of the prophenoloxidase (proPO) cascade, plays an essential role in the invertebrate immune response. It is triggered by minute amounts of microbial cell wall components leading to the activation of the enzyme proPO, which converts phenols into quinones and eventually resulting to the formation of melanin. Recent studies however, revealed that the importance of proPO in invertebrate immune response, in particular towards microbial pathogens, seems to be varied. Here, we discuss the function of proPO in penaeid shrimp, a commercially important aquaculture species, and its importance to shrimp survival.

Keywords: shrimp, prophenoloxidase (proPO), melanization


Corresponding author: Ikuo Hirono: hirono@kaiyodai.ac.jp
INTRODUCTION

A major and growing problem facing shrimp aquaculture is disease, which is largely due to the intensification of shrimp farming systems in response to the surging demand. Diseases caused by pathogenic organisms are responsible for heavy production losses in shrimp farms worldwide (Bondad-Reantaso et al., 2005). Most notable examples are Vibrio species and bacilliform viruses, such as the White Spot Syndrome Virus (WSSV), which were shown to cause mass mortalities within days after infection and can rapidly spread resulting in low survival rates (Lightner, 1996; Saulnier et al., 2000). There is, therefore, a need for a better understanding of the shrimp immune system in order to develop measures to lessen the effects of these diseases and hence to ensure the long term viability of the shrimp industry.

Although shrimp are devoid of an adaptive immune system, they possess an innate immune system that effectively protects them from harmful microorganisms (Lee and Söderhäll, 2002). This includes a rigid exoskeleton, the elimination of microbes through encapsulation, nodule formation or phagocytosis, release of antimicrobial peptides, clot formation, and melanization through the activation of the prophenoloxidase (proPO) cascade. The proPO cascade constitutes a major component of the shrimp humoral response and is triggered by very low amounts of bacterial cell wall components such as peptidoxyglycans, lipopolysaccharides and β-glucans (Hernández-López et al., 1996; Söderhäll and Cerenius, 1998). Here, we discuss the role of proPO in the shrimp immune response and its importance to the survival of penaeid shrimp.

ProPO activation

The proPO cascade in shrimp is set off in a stepwise process with the recognition of bacterial cell wall components by pattern recognition proteins. This process in turn, initiates the activation of a serine protease cascade that leads to the conversion of the proPO-activating enzyme (PPAE) to an active proteinase that converts the inactive enzyme precursor, proPO, into phenoloxidase (PO). PO, a copper containing oxidase, eventually catalyzes the oxidation of tyrosine to produce toxic quinone substances and other short-lived reaction intermediates that lead to the formation of melanin. It has been shown that melanin binds to the surface of bacteria and increase the adhesion of haemocytes to bacteria, thus accelerating their removal by nodule formation (Cerenius et al., 2008; da Silva, 2002). In shrimp, proPO has been shown to be localized in the haemocytes (Ai et al., 2009; Lai et al., 2005). In the last decade, many studies have investigated the various aspects of proPO in different shrimp species (Table 1).

ProPO function in shrimp

In invertebrates, the importance of proPO differs, particularly on their survival. In fruit flies, Drosophila melanogaster, proPO activation increases the effectiveness of other immune reactions (Tang et al., 2006) and mutant strains that are incapable of melanization tend to be more susceptible to infections (Braun et al., 1998; Lemaitre et al., 1995). In contrast, a separate study, using the same species, showed that proPO activation is not required against microbial infection (Leclerc et al., 2006). On the other hand in mosquitoes, proPO
Prophenoloxidase has a role in innate immunity in penaeid shrimp and melanization is altogether unimportant and is not required for their survival against some bacteria and microbes (Michel et al., 2006; Schnitger et al., 2007). In crayfish, a close relative of shrimp, proPO was reported to be essential for defense against *Aeromonas hydrophila* infections (Liu et al., 2007).

**Table 1**
Prophenoloxidase (proPO) studies in shrimp

<table>
<thead>
<tr>
<th>Species</th>
<th>Study</th>
<th>Author/s (Year)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Marsupenaeus japonicus</em></td>
<td>ProPO cloning, characterization</td>
<td>(Adachi et al., 1999)</td>
</tr>
<tr>
<td></td>
<td>Gene silencing</td>
<td>(Fagutao et al., 2009)</td>
</tr>
<tr>
<td><em>Penaeus monodon</em></td>
<td>Cloning and gene silencing</td>
<td>(Amparyup et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Cloning</td>
<td>(Sritunyalucksana et al., 1999)</td>
</tr>
<tr>
<td><em>Penaeus semisulcatus</em></td>
<td>Cloning and sequencing</td>
<td>unpublished</td>
</tr>
<tr>
<td><em>Penaeus vannamei</em></td>
<td>ProPO cloning, characterization</td>
<td>(Lai et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>Effect of temperature on proPO</td>
<td>(Pan et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Tissue distribution of proPO transcript</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ProPO characterization after Vibrio</td>
<td>(Yeh et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>alginolyticus infection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effect of lipopolysaccharides on proPO</td>
<td></td>
</tr>
<tr>
<td></td>
<td>expression</td>
<td>(Okumura, 2007)</td>
</tr>
<tr>
<td><em>Macrobrachium rosenbergii</em></td>
<td>Cloning, characterization</td>
<td>(Liu et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>Cloning</td>
<td>(Lu et al., 2006)</td>
</tr>
<tr>
<td><em>Penaeus californiensis</em></td>
<td>Activation of the proPO cascade</td>
<td>(Hernández-López et al., 1996)</td>
</tr>
<tr>
<td></td>
<td>Cloning</td>
<td>(Gollas-Galvan et al., 1999)</td>
</tr>
<tr>
<td></td>
<td>Effect of Calcium on proPO</td>
<td>(Gollas-Galván et al., 1997)</td>
</tr>
<tr>
<td><em>Fenneropenaeus chinensis</em></td>
<td>ProPO cloning, characterization</td>
<td>(Gao et al., 2009)</td>
</tr>
</tbody>
</table>

In shrimp, proPO was found to be required for defense against microbial pathogens. In Pacific white shrimp, *Litopenaeus vannamei*, proPO was shown to be involved in acute-phase immune defense against *Vibrio alginolyticus* and was also found to be regulated by ecdysone, a hormone that promotes growth and controls molting, which suggests that it may participate in other physiological processes (Yeh et al., 2009). Meanwhile, the expression
of two different forms of proPO in *L. vannamei* was found to be inhibited by WSSV infection (Ai et al., 2008; Ai et al., 2009). In Chinese shrimp, *Fenneropenaeus chinensis*, proPO expression increased after challenge with *V. anguillarum* (Gao et al., 2009). In black tiger shrimp, *Penaeus monodon*, gene silencing of proPO and its activating enzyme PPAE, resulted in a substantial reduction in total PO activity and increased susceptibility to *V. harveyi* infections (Amparyup et al., 2009; Charoensapsri et al., 2009). Gene silencing of proPO in kuruma shrimp, *Marsupenaeus japonicus*, resulted in increased bacterial counts in the haemolymph and increased mortality even in the absence of bacterial challenge (Fagutao et al., 2009). ProPO-depleted shrimp were also found to have lower haemocyte counts than control samples and to have significantly down-regulated expressions of antimicrobial peptides such as lysozyme, crustin and penaedin (Fagutao et al., 2009).

It is therefore apparent that, unlike flies and mosquitoes, proPO in shrimp plays an essential role in the innate immune response particularly against microbial pathogens and is important for shrimp survival. However, it is unclear whether proPO is involved in other physiological processes. Studies on how to increase or stimulate proPO expression in shrimp for application to actual farm conditions may help to improve the chances of survival during disease outbreaks.

**REFERENCES**


Amparyup, P., Charoensapsri, W. and Tassanakajon, A. 2009. Two prophenoloxidases are important for the survival of *Vibrio harveyi* challenged shrimp *Penaeus monodon*. *Developmental and Comparative Immunology* 33: 247-56.


Prophenoloxidase has a role in innate immunity in penaeid shrimp


