

## 論文の内容の要旨

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論文題目 Physiological studies on the mechanisms of immune response and reproductive development in penaeid shrimp  
(クルマエビ類における免疫反応と性成熟機構に関する生理学的研究)

### **General Introduction**

The annual production of aquacultured shrimp has been over 3 million tons during the past five years, and continues to increase (FAO, 2010). Three penaeid shrimps, *Penaeus monodon*, *Fenneropenaeus chinensis*, and *Litopenaeus vannamei* accounted for over 86% of total production in 2002. However, the shrimp aquaculture industry harbors severe impediments, such as the outbreak of viral disease and difficulties in inducing reproduction in capacity. Eystalk ablation is the major means of inducing ovarian development in female shrimp, but it lead to deterioration in spawn quality and quantity over time, in addition to increasing the possibility of infection. Hence, it is important to conduct research relating to the biology and physiology of animals used in aquaculture operations where it is necessary to both control disease and induce reproductive development. The improvement of immune activity may be an effective strategy for the control of shrimp disease (Bachere, 2000). Therefore, physiological studies linking immune response and reproductive development in penaeid shrimp is considered to be of value, and for this reason, studies on *Fenneropenaeus chinensis* and *Litopenaeus vannamei* were undertaken.

## **Chapter 1. Molecular cloning of prophenoloxidase in hemocytes of the fleshy shrimp, *Fenneropenaeus chinensis***

In the crustaceans, the first step in immune defense is the detection of invading non-self molecules. This is accomplished by the prophenoloxidase activating system (proPO-AS). Firstly, cDNA encoding proPO of *F. chinensis* (f-proPO) was obtained from hemocytes; it had a full length of 3023 bp, with an open reading frame (ORF) of 2061 bp, a 105-bp 5'-untranslated region, and a 906-bp 3'-untranslated region containing the poly A signal. This 3023-bp f-proPO cDNA encoded a putative protein of 686 amino acids having a molecular mass of 78.1 kDa, and high sequence similarity to proPOs of *P. monodon* (93%), *Penaeus semisulcatus* (92%), *L. vannamei* (88%), and *Macrobrachium rosenbergii* (80%). These results were utilized in subsequent experiments to elucidate the actions of proPO-AS, and the effects of  $\beta$ -glucan or rutin in immune response in *F. chinensis*.

## **Chapter 2. Effects of $\beta$ -glucan and rutin on immune responses in hemocytes of the fleshy shrimp, *Fenneropenaeus chinensis***

In several shrimp species,  $\beta$ -glucan and rutin are considered to be immunostimulants that affect to immune response. In order to investigate the effects of dietary  $\beta$ -glucan and rutin as immunostimulants in the absence of pathogen challenge, *F. chinensis* were fed with  $\beta$ -glucan (0.5 or 1 g kg<sup>-1</sup> diet) or rutin (0.5 or 1 g kg<sup>-1</sup> diet) for 10 days. Total hemocyte count (THC) and the expression of four immune-related genes, those for prophenoloxidase (proPO), peroxinectin (PX), lipopolysaccharide and  $\beta$ -glucan binding protein (LGBP), and c-type lectin (CL), were determined in hemocytes. The THCs of shrimp fed  $\beta$ -glucan at 1 g kg<sup>-1</sup> diet, and rutin at 1 g kg<sup>-1</sup> diet were significantly higher than those of the control ( $P < 0.05$ ). The expression of proPO mRNA was slightly downregulated, and that of LGBP mRNA was upregulated (except in shrimp fed rutin at 1 g kg<sup>-1</sup> diet). PX and CL mRNA remained constitutively expressed in all groups. The results of this chapter revealed that  $\beta$ -glucan and rutin dietary supplements have minimal effect on immune response in the absence of pathogen challenge, but that rutin is a possible candidate immunostimulant for *F. chinensis*.

## **Chapter 3. Effects of cyclic nucleotides and a calcium ionophore on vitellogenin mRNA expression using ovarian incubation in *Litopenaus vannamei***

In crustaceans, vitellogenesis is considered to be under the negative control of the VIH, a neuropeptide in the crustacean hyperglycemic hormone (CHH) family that is secreted from the X-organ/sinus gland complex in the eyestalks. The actions of peptide hormones are thought to be mediated by signaling pathways such as cyclic nucleotides and Ca<sup>2+</sup> in the target organ cells such as ovary and hepatopancreas tissue. The incubation of ovarian fragments was used to investigate whether cyclic nucleotides and Ca<sup>2+</sup> are involved in the regulation of Vg mRNA levels in the ovary of *L. vannamei*. There were no significant differences in Vg mRNA levels when cAMP and cGMP analogues were applied in incubation in dosages ranging from 0.1 to 1000  $\mu$ M. These results were inconclusive, and made it necessary to further investigate how signaling pathways based on peptide hormones controls vitellogenesis in adult female shrimp.

#### **Chapter 4. Studies on membrane guanylyl cyclase as a crustacean hyperglycemic hormone (CHH) family receptor in *Litopenaus vannamei***

It was considered possible that the action of peptide hormones may be mediated by receptor guanylyl cyclase, a single-pass transmembrane protein. To investigate whether membrane guanylyl cyclase (mGC) is involved in the regulation of *Vg* mRNA levels, as a first step, the cDNA sequence of mGC in the ovary of *L. vannamei* is currently being determined. In order to perform the cloning of this receptor, the specific primers for mGC were designed from a cDNA library, and used for subcloning. Subsequently, mGC mRNA expression will be examined in order to investigate its distribution in somatic tissues. The results of this chapter should shed light on whether a receptor guanylyl cyclase is involved in cellular signaling in vitellogenesis in female shrimp.

#### **Chapter 5. Alterations of pattern in immune response and vitellogenesis during induced ovarian development by unilateral and bilateral ablation in *Litopenaus vannamei***

Eyestalk ablation is often used to induce ovarian development in female shrimp, but it alters the animal's hormonal balance, and affects its physiology and immune system. Therefore, in this chapter, alterations in patterns of both immune response and *Vg* levels induced by eyestalk ablation were investigated. Following bilateral ablation, GSI and *Vg* mRNA levels started to increase after 4 days, and reached peak levels 10 days after ablation. After 20 days, levels became somewhat decreased. Oocyte size increased following ablation; ovaries were at the endogenous vitellogenic stage 10 days later, and at the exogenous vitellogenic stage 20 days later. Analysis of immune-related genes is currently in progress, and primers for real-time PCR have already been designed. The results of this chapter are expected to clarify whether immune response is altered by eyestalk ablation, and whether induced reproduction potentially leads to greater chance of infection.

#### **General Discussion**

To establish strategies for the control of disease and develop alternatives to eyestalk ablation in order to achieve more sustainable shrimp aquaculture, it is important to accumulate knowledge from physiological studies concerning shrimp immunology and endocrinology. This study was an attempt to better understand physiological mechanisms in terms of both immune response and reproductive development in the penaeid shrimps, *F. chinensis* and *L. vannamei*. Therefore, this study has value for creating a new approach to integrating knowledge of gonadal maturation and shrimp immunity, and thus is expected to lead to a better understanding of shrimp physiology.

#### **References**

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