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Comparative Biochemistry and Physiology, Part B 142 (2005) 209 – 216
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# Starvation and diet composition affect mRNA levels of the high density-lipoprotein-β glucan binding protein in the shrimp *Litopenaeus vannamei*

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Received 17 March 2005; received in revised form 29 June 2005; accepted 6 July 2005
Available online 19 August 2005

**Abstract**

A high density lipoprotein-beta glucan binding protein (HDL-BGBP) is synthesized in the hepatopancreas of the white shrimp *Litopenaeus vannamei* and secreted to the hemolymph. Recently, we reported the HDL-BGBP full length cDNA sequence and found that the predicted polypeptide is larger than the mature protein and also, that it contains a long 5′- and 3′-UTRs that may be involved in transcript level regulation. To test whether starvation and feeding may play a role in regulating HDL-BGBP mRNA levels, two different stimuli were evaluated: starvation and composition of diets. After 24 h, the steady state HDL-BGBP mRNA levels of starved shrimp decreased, suggesting that synthesis of the lipoprotein is less required in the absence of food. When shrimp were fed with diets containing different concentrations of protein and lipids, changes in HDL-BGBP mRNA levels were also detected. Shrimp fed the lower concentration of protein and lipid feed accumulated higher levels of HDL-BGBP mRNA. These results indicate that feeding influences HDL-BGBP transcript levels in the hepatopancreas.


*Keywords:* Beta glucan binding protein; Diet; HDL-BGBP; Hepatopancreas; Lipoprotein; mRNA levels; Shrimp; Starvation

## 1. Introduction

The HDL-BGBP from the white shrimp *Litopenaeus vannamei* has two main physiological roles: 1) transport of lipids (Yepiz-Plascencia et al., 1995; Ravid et al., 1999), and, 2) a particle recognition protein (PRP), a component of the immune system (Vargas-Albores et al., 1996). In the crayfish *Pacifastacus leniusculus*, this plasma protein was first identified as a β-glucan binding protein (BGBP) that forms a complex with β-glucans, inducing spreading and degranulation of hemocytes and activation of the prophenoloxidase (proPo) system (Barracco et al., 1991) and afterwards, it was identified as a lipoprotein (Hall et al., 1995). The HDL-BGBP is synthesized in the hepatopancreas of *L. vannamei,* as was shown in juvenile shrimp using an in vitro cell-free translation system and immunodetection (Yepiz-Plascencia et al., 2000b) and RT-PCR (Romo-Figueroa et al., 2004). No transcript was detected in hemocytes, indicating that the hepatopancreas is the source of the plasma circulating lipoprotein, as was previously shown for *P. leniusculus* (Cerenius et al., 1994).

We recently reported the full length cDNA sequence of the HDL-BGBP from *L. vannamei* (GenBank accession no. AY249858). It is a longer than anticipated 6 Kb transcript (6379 bp) coding for 1454 amino acid residues and a predicted 141 kDa precursor protein, with no clear secretion signal peptide sequence. It also contains two potential subtilisin-like processing sites near the mature N- and C-termini, suggesting a highly processed mature protein

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doi:10.1016/j.cbpc.2005.07.005

(Romo-Figueroa et al., 2004) that is also glycosylated (Yepiz-Plascencia et al., 1995). Furthermore, the full length cDNA contains long 5′ and 3′-untranslated regions with as yet, unknown functions.

Although insect hemolymph lipoproteins have been extensively studied (Van der Horst, 1990; Ryan and Van der Horst, 2000; Arrese et al., 2001), the lipid transport mechanism, and the regulation of gene expression of the crustacean lipoproteins are still unknown. Nevertheless, the dual role, nutrition and defense, of HDL-BGBP makes it an interesting molecule to investigate gene expression. Bifunctional hemolymph proteins as HDL-BGBP and VHDL-CP have been reported from shrimp (Yepiz-Plascencia et al., 1998; Yepiz-Plascencia et al., 2002), and crayfish species (Komatsu et al., 1993; Hall et al., 1995).

Starvation effects have been used to understand mechanisms leading to acute-responses generated by imbalance in energy homeostasis. Starvation changes gene expression of several genes in vertebrates. For example, after 48 h of starvation in rats, the expression of more than 54 genes increased, including proteins involved in energy and protein metabolism, stress response, signal transduction and nutrient transporters and receptors. Interestingly one of these is the apolipoprotein B-100, a component of the plasma LDL (Zhang et al., 2001). Starvation also causes acute responses in crustaceans, affecting metabolism, growth, and the biochemical composition of the hepatopancreas in shrimp species (Cuzon et al., 1980; Barclay et al., 1983; Muhlia-Almazán et al., 2003; Sánchez-Paz et al., 2003). Crustaceans are constantly challenged by several stressors, including endogenous (as molting) or exogenous agents (temperature and salinity changes, pathogens, predators, food, etc.). A timely response to these threats is critical for survival. The present study was designed to evaluate the effect of starvation and diet composition on the HDL-BGBP mRNA levels.

## 2. Materials and methods

Two separate bioassays were conducted to evaluate the effect of: i) starvation, and ii) diets containing different levels of protein and lipids. Juvenile *L. vannamei* shrimp, weighing 9.0±1.0 g, were obtained from culture ponds at CIBNOR (Mexico) and maintained under controlled laboratory conditions. During a 15-days acclimatization period, shrimp were placed in 120 L plastic tanks in filtered marine water at 28 °C, and 34 ppt salinity, and were fed twice daily with commercial feed (Silvercup™, El Pedregal, Mexico, under license of Sterling H. Nelson and Sons, Inc.). Uneaten food and solid excreta were discarded daily. All shrimp used in the assays were previously selected at intermolt stage by setogenesis, by observing the changes in the seta of the inner margin of uropods (Chan et al., 1988).

### 2.1. Starvation assay

This assay was designed to evaluate the effect of starvation on the HDL-BGBP mRNA steady state levels in the hepatopancreas of shrimp. At the end of the acclimatization, after being selected by molting stage, shrimp were fed commercial food (Silver cup containing 46% protein and 7.2% lipids, according to the producers) and then sampled at 2, 24, 72 and 120 h after the last feeding. Seven replicates of each time point were collected. Shrimp were decapitated; the hepatopancreas was dissected, individually weighed and stored at −80 °C until used.

### 2.2. Diets with different content assay

After acclimatization, three groups of shrimp were randomly chosen and separated in plastic tanks. Three isocaloric diets were prepared: diet 1, 2, and 3, with different protein and lipid content as shown in Table 1 (being the protein content, the indicator of quality of a diet, as commonly assumed in marine cultured animals). The diets were designed and formulated using the Mixit-winx computer software (Agricultural Software Consultants, Inc., USA). A proximal analysis was done by triplicate for each diet. Statistical analyses between the three diets replicates found no statistical differences between the three energy values of the experimental diets. Shrimp of each tank (9), were fed with one of the experimental diets, twice a day ad libitum during 3 weeks. Once shrimp were acclimated to a diet, six replicates of each experimental trial were collected after ~2 h of feeding, selected at inter-molt stage, individually weighed, decapitated and the hepatopancreas was extracted and frozen at −80 °C until use.

### 2.3. HDL-BGBP mRNA levels by semi-quantitative RT-PCR

Total RNA was isolated from the hepatopancreas of each shrimp individually using TRIzol® reagent (GIBCO-BRL, New York). Purity and concentration of the isolated RNA were examined by $A_{260}/A_{280}$ ratios and analyzed on a 1.2% agarose–formaldehyde gel and ethidium bromide staining

Table 1
Experimental diets composition

| Feed label | Wet (%) | Protein content (%) | Lipids (%) | Ash (%) | Crude fiber (%) | NFE** | Energy (Cal/g) |
|---|---|---|---|---|---|---|---|
| 1 | 14.3±0.2 | 15.2±0.20 | 3.06±0.08 | 5.82±0.09 | 2.77±0.07 | 73.11 | 4562±13 |
| 2 | 12.7±0.05 | 31.8±0.02 | 4.49±0.07 | 6.41±0.1 | 5.79±0.09 | 51.49 | 4461±8.8 |
| 3 | 17.2±0.08 | 49.8±0.20 | 5.65±0.21 | 10.6±0.21 | 6.26±0.24 | 27.59 | 4602±24 |

*Protein main source was fish meal (70.3% protein). **Nitrogen free extracts, considered mainly as carbohydrates. Each value represents mean±S.D ($n$=3).