Comparative Biochemistry and Physiology, Part A 164 (2013) 327–332

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Neuroendocrine responses of a crustacean host to viral infection: Effects of infection of white spot syndrome virus on the expression and release of crustacean hyperglycemic hormone in the crayfish *Procambarus clarkii*

Ling-Jiun Lin [a], Yan-Jhou Chen [a], Yun-Shiang Chang [b], Chi-Ying Lee [c,*]

[a] Department of Biology, National Changhua University of Education, Changhua 50058, Taiwan
[b] Department of Molecular Biotechnology, Da-Yeh University, Changhua 51591, Taiwan
[c] Graduate Institute of Biotechnology, National Changhua University of Education, Changhua 50058, Taiwan

ARTICLE INFO

Article history:
Received 9 September 2012
Received in revised form 13 November 2012
Accepted 14 November 2012
Available online 19 November 2012

Keywords:
White spot syndrome virus
Crustacean hyperglycemic hormone
Carbohydrate metabolism
Glycolysis

ABSTRACT

The objectives of the present study were to characterize the changes in crustacean hyperglycemic hormone (CHH) transcript and peptide levels in response to infection of white spot syndrome virus (WSSV) in a crustacean, *Procambarus clarkii*. After viral challenge, significant increase in virus load began at 24 h post injection (hpi) and the increase was much more substantial at 48 and 72 hpi. The hemolymph CHH levels rapidly increased after viral challenge; the increase started as early as 3 hpi and lasted for at least 2 d after the challenge. In contrast, the hemolymph glucose levels did not significantly changed over a 2 d period in the WSSV-infected animals. The CHH transcript and peptide levels in tissues were also determined. The CHH transcript levels in the eyestalk ganglia (the major site of CHH synthesis) of the virus-infected animals did not significantly change over a 2 d period and those in 2 extra-eyestalk tissues (the thoracic ganglia and cerebral ganglia) significantly increased at 24 and 48 hpi. The CHH peptide levels in the eyestalk ganglia of the virus-infected animals significantly decreased at 24 and 48 hpi and those in the thoracic ganglia and cerebral ganglia remained unchanged over a 2 d period. These data demonstrated a WSSV-induced increase in the release of CHH into hemolymph that is rapid in onset and lasting in duration. Changes in the CHH transcript and peptide levels implied that the WSSV-induced increase in hemolymph CHH levels primarily resulted from an enhanced release from the eyestalk ganglia, but the contribution of the 2 extra-eyestalk tissues to hemolymph pool of CHH increased as viral infection progressed. The combined patterns of change in the hemolymph glucose and CHH levels further suggest that the virus-enhanced CHH release would lead to higher glycolytic activity and elevated glucose mobilization presumably favorable for viral replication.

1. Introduction

White spot disease (WSD) is a highly contagious and lethal viral disease of penaeid shrimps caused by an infection of white spot syndrome virus (WSSV), which is an enveloped, rod-shaped virus containing a double-stranded DNA genome. WSSV has a wide range of potential hosts including more than 90 species of arthropods. It is highly virulent and leads to extremely high mortality rates within days in the case of cultured shrimp populations. Thus, it poses serious threats in particular to shrimp aquaculture, causing tremendous loss to the global farming industry since the first documented disease outbreak in the early 1990 (Sánchez-Paz, 2010).

Although therapeutic measures for effective control of WSSV infection are currently unavailable, much has been learned about WSSV regarding its morphology, ultrastructure, genome, gene expression pattern, and mode of transmission, etc. (see Escobedo-Bonilla et al., 2008; Leu et al., 2009; Sánchez-Paz, 2010). Despite the host–virus interaction has been one of the recent focuses in understanding WSSV pathogenesis (Jiravanichpaisal et al., 2006; Leu et al., 2007, 2012; Wang et al., 2007; Rodríguez et al., 2012), the responses of the host endocrine system to WSSV infection have yet to be characterized.

Crustacean hyperglycemic hormone (CHH) is a polypeptide hormone originally identified in an important crustacean neuroendocrine tissue, the X-organ/sinus gland complex, located within the eyestalks (see Keller, 1992; Soyez, 1997); more recent studies have firmly established that CHH is also expressed in several other extra-eyestalk neuroendocrine tissues (see Webster et al., 2012). CHH belongs to a family of polypeptide hormones, the CHH family (Kegel et al., 1991; Soyez, 1997), which also includes molt-inhibiting hormone (MIH), vitellogenesis-inhibiting hormone (VIH), and mandibular organ-inhibiting hormone (MOIH), and insect ion transport peptide (ITP) (Chen et al., 2005). Recent studies have expanded the existence of the CHH family peptides beyond arthropods to ecdysozoans (Christie, 2008; Montagné et al., 2010).

* Corresponding author at: Graduate Institute of Biotechnology, National Changhua University of Education, 1 Jin Der Road, Changhua 50058, Taiwan. Tel.: +886 4 724 7756; fax: +886 4 721 1156.
E-mail address: bicylee@cc.ncue.edu.tw (C.-Y. Lee).

1095-6433/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cbpa.2012.11.009

Functionally, CHH plays regulatory roles in the main pathways of carbohydrate metabolism (see Santos and Keller 1993) and is considered a stress hormone that is critically involved in eliciting stress-induced hyperglycemia (Webster, 1996; Chang et al., 1998, 1999; Zou et al., 2003; Lorenzon et al., 2004; Webster et al., 2012). In light of the observations that viral infection is usually associated with changes in carbohydrate metabolism in mammalian hosts (see Yu et al., 2011; Noch and Khalili, 2012), it was reasoned that CHH would probably constitute an important part of the endocrine responses to viral infection in crustaceans and the responses might bear significant implication for understanding viral pathogenesis and identifying potential targets for therapeutic intervention. As a first effort in characterizing the endocrine responses to WSSV infection, the aims of the present study were to determine the effects of WSSV infection on a crustacean host *Procambarus clarkii*, specifically the effects on CHH expression in various neuroendocrine tissues and its release into the circulation.

2. Materials and methods

2.1. Animals

Animals (*P. clarkii*) were purchased from local fisherman and maintained in the laboratory as previously described (Zou et al., 2003). Animals used in the present study were intermolt adults that had been determined to be WSSV-free using a polymerase chain reaction (PCR)-based diagnostic kit IQ2000™ (GeneReach Biotechnology Corp.) according to the manufacturer's protocol.

2.2. Virus and viral challenge

The viral preparations (WSSV-TW strain, GenBank accession no. AF440570) were kindly provided by the Core Facilities for Shrimp Functional Genomics (SFGC) (http://shrimpwssv.lifescience.ntu.edu.tw). The WSSV-TW strain (Chang et al. 2008) originates from a batch of WSSV-infected *Penaeus monodon* collected in Taiwan in 1994 (Wang et al. 1995; Lo et al. 1999). The preparations were stored at −80 °C and before use diluted with sterile 0.01 M phosphate-buffered saline (PBS) and filtered through a 0.45 μm filter.

Before experimental procedures, animals were deprived of food for 1 d. They were then each injected with 100 μl of diluted viral solution (2×10^6 particles/animal) or 0.01 M PBS (as vehicle controls) using an insulin syringe coupled to a 29-gauge needle (Terumo Medical Corp., Elkton, MD). Tissues and hemolymph samples (see below) were collected from animals before injection (taken as time zero) and at designated time points thereafter for subsequent analyses.

2.3. Absolute quantification of virus load by real-time polymerase chain reaction (PCR)

A real time PCR assay for viral ICP11 gene (GenBank accession numbers HM778020) (Chang et al., 2011) was employed for absolute quantification of the WSSV copy number in the experimental animals (see Section 2.2.). The genomic DNA was extracted from the pleopods of the animals using the reagents supplied with the IQ2000™ kit and the extracted samples were amplified using a commercially available kit (LightCycler FastStart DNA Master SYBR Green I, Roche) in a 10-μl reaction according to the manufacturer's protocol. Reactions were performed under the following conditions: an initial denaturation (10 min, 95 °C), 40 cycles of denaturation (10 s, 95 °C), annealing (7 s, 55 °C), and extension (13 s, 72 °C). Primers used were ICP11-F-real and ICP11-R-real (see Table 1). Standard curves were constructed by plotting the copy number of ICP11 gene (pGEM/ICP11, Chang et al., 2011) vs. the threshold cycle (C_t) value of the PCR reaction (see Fig. 1).

Table 1
Sequences of primers used in the present study for the real-time quantitative polymerase chain reactions.

Primer	Sequence (5′ to 3′)	Accession number
ICP11-F-real	AGGCAGTCAGGAAGAGTGATCTAGA	HM778020
ICP11-R-real	AATTCTTCGATGCCTCCATTGA	
CHHe2F	AACCTCTCAGCTTCCTCTCCCAAG	AB027291
SG-R-400-427	CATAGCAGTTTTGTCTGCAGGTGGTGGC	
GAPDH-F-209	TCATGGTGTGTTCAAGGGT	AB094145
GAPDH-R-361	AGAGGCTTTCTCAATAGTGG	

2.4. Relative quantification of the levels of CHH transcript by real-time PCR

The levels of CHH transcript in tissues were measured by a real-time PCR assay. Tissues (eyestalk ganglia, thoracic ganglia, and cerebral ganglia) dissected from experimental animals (see Section 2.2.) were processed for total RNA extraction and reverse transcription according to described protocols (Tsai et al., 2008).

For real-time PCR, cDNA templates were amplified in a 10-μl reaction using the same LightCycler reagents mentioned above. Reactions were performed under the following conditions: an initial denaturation (10 min, 95 °C), 50 cycles of denaturation (10 s, 95 °C), annealing (7 s, 60 °C), and extension (14 s, 72 °C); the rate of temperature change was 20 °C/s. The primers used were CHHe2F and SG-R-400-427 for PCR amplification of *CHH* (see Table 1). The forward primer (CHHe2F) covers a sequence encoding the 21st to 28th residues of the CHH-precursor related peptide, a stretch that is common in sequence to both the CHH and CHH-like (CHH-L) precursors (Wu et al., 2012a). The specificity of the PCR for CHH relies on the backward primer (SG-R-400-427, see Table 1), which covers a continuous sequence spanning from exon II to exon IV of CHH transcript (that is composed of exons I, II, and IV). Thus, amplification using SG-R-400-427 together with the forward primer would be expected to specifically amplify the transcript encoding CHH, but not the CHH-L transcript (that is composed of exons I, II, III and IV) (Wu et al., 2012a). Sequence analysis of the sole PCR product amplified using the primer pair confirmed that the product encodes CHH, not CHH-L (data not shown). Primers used for amplification of the reference gene glyceraldehyde-3 phosphate dehydrogenase (*GAPDH*) were GAPDH-F-209 and GAPDH-R-361 (see Table 1). Glyceraldehyde-3 phosphate dehydrogenase gene was selected as the reference gene on the ground that preliminary tests indicated that for each examined tissue the threshold cycle value for its

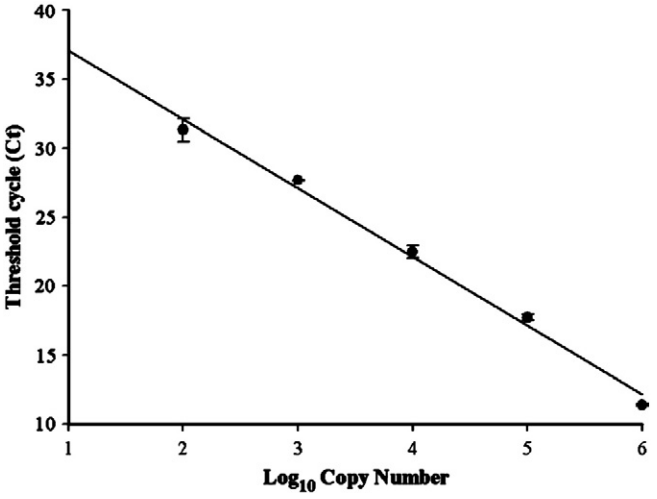

Fig. 1. A typical standard curve for quantification of the viral ICP11 gene by a real-time PCR. A series of diluted plasmid DNA samples (pGEM/ICP11) with a range of 1×10^2–1×10^6 copies of ICP11 was real-time PCR-amplified. The threshold cycle (C_t) of each reaction was plotted against the log-transformed copy number. (n = 5 for each data point).