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Molecular cloning of kuruma shrimp *Marsupenaeus japonicus* phosphopyruvate hydratase and its role in infection by white spot syndrome virus and *Vibrio alginolyticus*

Baozhen Sun [1], Zhi Wang [1], Fei Zhu *

College of Animal Science and Technology, Zhejiang Agriculture and Forestry University, Hangzhou 311300, China

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ABSTRACT

Phosphopyruvate hydratase (enolase) is a housekeeping gene for energy production in many eukaryotes. The 1914-base pair (bp) cDNA sequence of phosphopyruvate hydratase was obtained from the muscles of *Marsupenaeus japonicus* using RT-PCR and RACE. The results suggested that enolase is highly expressed in the muscle of *M. japonicus* but not in other organs. We compared the nucleotide sequence of enolase in *M. japonicus* to that in other species and found 93, 49.08, 47.1, and 44.62% homology to the enolase of *Penaeus monodon*, *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Danio rerio*, respectively. Furthermore, the amino acid sequence of *M. japonicus* showed 97, 77, 73, and 69% homology to the corresponding sequences in the other four species, respectively. We also found that white spot syndrome virus (WSSV) or *Vibrio alginolyticus* infection dramatically altered the expression level of enolase. Knockdown of enolase resulted in a significant up-regulation ($P < 0.01$) of immune-related genes such as *L-lectin*, mitogen-activated protein kinase (*MAPK*), nitric oxide synthase (*NOS*), prophenoloxidase (*proPO*), and signal transducer and activator of transcription (*STAT*), and a significant down-regulation of *p53* and *TNF* ($P < 0.01$) in the muscle of shrimp. We first used RNAi to demonstrate that enolase is very important for WSSV infection of kuruma shrimp, and reported that the time of death was delayed in WSSV-infected shrimp. Characterization of *M. japonicus* enolase will enable us to further study the role of this enzyme on the immune function in shrimp.

Statement of relevance

Phosphopyruvate hydratase (enolase) is a housekeeping gene for energy production in many eukaryotes. The 1914 bp cDNA sequence of phosphopyruvate hydratase was obtained from the muscles of shrimp *Marsupenaeus japonicus*. The results suggested phosphopyruvate hydratase was highly expressed in the muscle transcriptome of *M. japonicus* but not in other organs. We also found that white spot syndrome virus (WSSV) or Vibrio *alginolyticus* infection will changed phosphopyruvate hydratase expression tempestuously after the challenge. When phosphopyruvate hydratase was knock down, immune genes like *L-lectin*, *MAPK*, *NOS*, *proPO* and *STAT* were up-regulated significantly ($P<0.01$), but *p53* and *TNF* were down-regulated significantly ($P<0.01$) in the muscle of shrimp. We firstly demonstrated that phosphopyruvate hydratase is very important for the progress of WSSV infection and the dead time of WSSV-infected shrimp was significantly delayed by the RNAi of phosphopyruvate hydratase. Characterization of *M. japonicus* phosphopyruvate hydratase will enable us to further study on the role of phosphopyruvate hydratase on immune function in shrimp. Our study is very related to the antiviral immunity of shrimp and belong to the disease of aquaculture.

1. Introduction

Phosphopyruvate hydratase, also known as 14-3-2 protein, 2-phosphoglycerate dehydratase or enolase, is involved in glycolysis and catalyzing the conversion of 2-phosphoglycerate to phosphoenolpyruvate (Bruns and Gerald, 1976). A previous study has reported that the expression of this metabolic enzyme is increased in blood cancer cells (Lopez-Pedrera et al., 2006). Another study found that its expression is decreased in ovarian cancer cells treated by antitumor drug (Li et al., 2005). These reports suggest that NaBu, which is a well-known antitumor agent, down-regulated the expression of enolase and hindered cell growth. Enolase is a housekeeping gene related to energy

* Corresponding author.
E-mail address: zhufei@zju.edu.cn (F. Zhu).
[1] Same contributions to this work.

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Table 1
Universal and specific primers used in this study. Phosphopyruvate hydratase was marked as PH.

Primer name	Nucleotide sequence (5′ →3′)	Purpose
3′ RACE GSP	ATGGACGTGGCTGCTTCCGAGTT	PH first primer for 3′′RACE
3′ RACE NGSP	GCAGATCACTGGGGACCAACTT AGG	PH second primer for 3′′RACE
5′′RACE sp1	TCTTGGTCCAGTTCTCCCAGTCG	PH first primer for 5′′RACE
5′′RACE sp2	AGACAATGGGGAACTCGTTGCAGA	PH second primer for 5′′ RACE
5′′RACE sp3	TTGTAGAACTCGGAAGCAGCCAC	PH third primer for 5′′RACE
PH realtime-F	GACGGCACTGAGAACAAGAGC	For PH expression
PH realtime-R	AGCCACCATTGATGACATTGAAG	For PH expression
β-Actin-F	CGAGCACGGCATCGTTACTA	For β-actin expression
β-Actin-R	TTGTAGAAAGTGTGATGCCAGA TCT	For β-actin expression
Hemocyanin-F	AACCCTGAACAAAGAGTTGCCTAT	For hemocyanin expression
Hemocyanin-R	AACGGACGGTAAGTTGATGATGT	For hemocyanin expression
IMD-F	ATTCATCCGTCTACCTCCCTACA	For IMD expression
IMD-R	GAGCTGAGTCTGTCTTAATGTTAT CC	For IMD expression
L-type lectin-F	ATGTTATGCCATCTGCCTCGTATTT	For L-lectin expression
L-type lectin-R	CTTTCGCTGCTGCTCTTTCTGTT	For L-lectin expression
MAPK-F	CGCATCACTGTTGAGGAGG	For MAPK expression
MAPK-R	GCAGGTCATCAAGTTCCATCT	For MAPK expression
NOS-F	CCAGGATCTTCTTGTTGGTGTTG	For NOS expression
NOS-R	CCCTCATCTGTAGCATAAAGTTCTC	For NOS expression
p53-F	TTCCTGCCTGGCTGACTCTA	For p53 expression
p53-R	CACCCAATCTTCCAACATCACAT	For p53 expression
proPO-F	TTCTACCGCTGGCATAAGTTTGT	For proPO expression
proPO-R	TATCTGCCTCGTCGTTCCTCAC	For proPO expression
STAT-F	TGGCAGGATGGATAGAAGACAAG	For STAT expression
STAT-R	TGAATAAGCTGGGATACGAGGGA	For STAT expression
TNF-F	ACAGACGGTCCAGAGTCCCAAAG	For TNF expression
TNF-R	GCGACGAAGTGAGCCACAGTAA	For TNF expression
PH-siRNA-362	GCCTTGGTG CTAATGCCAT	For PH RNAi
PH-siRNA-809	GCGAGAACATCCACGACTT	For PH RNAi
PH-siRNA-1125	CCTTCT GGCCAAGAAGAAT	For PH RNAi

production, and it shows obvious changes to hypoxic stress in shrimp (Boonchuoy et al., 1999). Another study reported that NaBu treatment could decrease the enolase expression level in *Fenneropenaeus chinensis* by over twofold (Jiang et al., 2009). Enolase was also found to act as a novel IgE reactive protein in prawn (Tomm et al., 2013).

Recent studies have verified that enolase activates plasminogen, is involved in the processes of parasite infection and migration (Fox and Smulian, 2001; Bao et al., 2014), and contributes to parasite survival by preventing parasites from the immune attack of the host (Liu et al., 2012; Nogueira et al., 2012). Current advances in research have demonstrated the diagnostic potential of enolase parasitic diseases, as well as its importance in drug development and as a vaccine target (Chen et al., 2012; Manneck et al., 2012). Furthermore, the important role of enolase in other microbes has already been established. Viruses do need to utilize host cell components in their infection but bacteria do not need to use host cell components as bacterial enolase exists. The DNA methyltransferase of the *Escherichia coli* bacteriophage T1 appears to interact with *E. coli* enolase upon infection (Gassner et al., 1998). Recently, some research reports have showed that enolase is critical for infection and enhances bacterial survival. *Vibrio parahaemolyticus* enolase, which has plasminogen-binding activity, is an adhesion-related factor and plays an important role in pathogenicity (Jiang et al., 2014). As collagen is a target of pathogens for adhesion, colonization, and invasion of host tissue, enolase can act as a collagen-binding protein in *Lactobacillus plantarum* (Salzillo et al., 2015). *Streptococcus canis* enolase has shown to significantly enhance bacterial survival in phagocytosis analyses using human neutrophils (Fulde et al., 2013). In fungi, the extracellular enolase of *Candida albicans* has been shown to mediate colonization on its primary translocation site. As enolase functions as a glycolytic enzyme and extracellular peptide, it is a remarkable example of gene sharing in fungi (Silva et al., 2014). In invertebrates, host enolase has been reported to be involved in viral infection. *Bombyx mori* enolase may play an important role in intracellular transportation

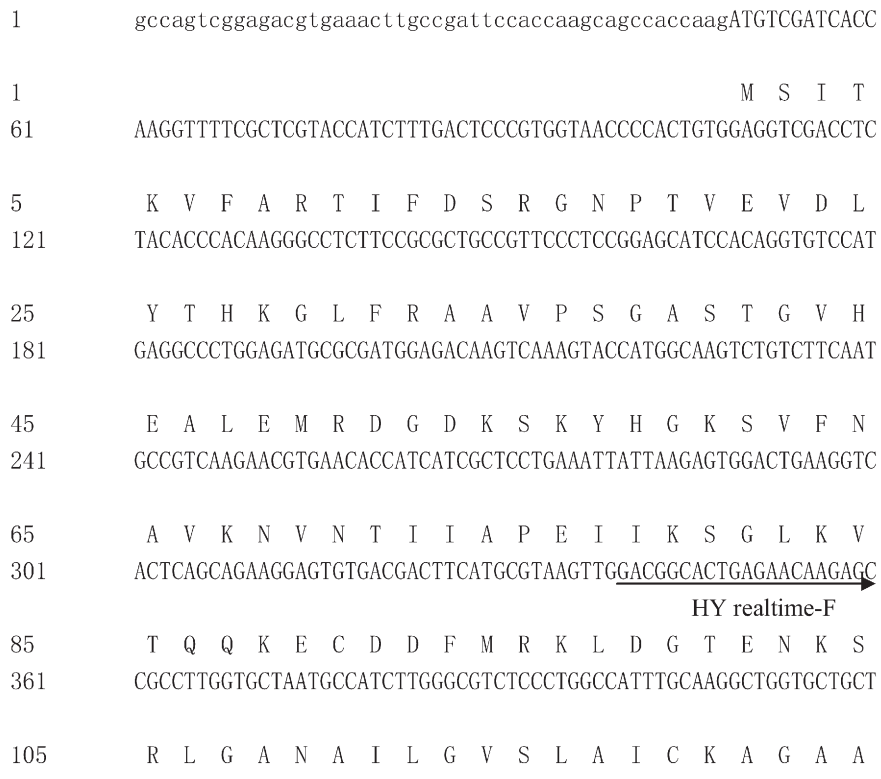

Fig. 1. Nucleotide and deduced amino acid sequences of enolase. The nucleotide sequence is displayed in the 5′–3′ direction and numbered at the left. The deduced amino acid sequence is shown in a single capital letter amino acid code. The 3′-untranslated region (UTR) and 5′-UTR are shown with lowercase letters. Codons are numbered at the left with the methionine (ATG) initiation codon, and an asterisk denotes the termination codon (TAA). Rapid amplification of cDNA ends (RACE) and real-time qPCR primers are marked with arrows.