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Molecular Identification and Expression Analysis of a Novel Cyclophilin A Gene in the Red Swamp Crayfish, *Procambarus clarkii*

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Abstract: Cyclophilin A (Cyp A) is the main intracellular receptor of cyclosporin A (CsA) belonging to the immunophilin family, which is known as an effective immunosuppressive drug. This study aimed to gain insights into the structure and biological function of cyclophilin A in the red swamp crayfish, Procambar*us clarkii* (PcCypA). We cloned PcCypA by homology cloning and anchored polymerase chain reaction (PCR), and assessed its mRNA and protein expression levels in different tissues using quantitative real-time PCR and western blot analysis, respectively. The full-length DNA contained a 5′ untranslated region (UTR) comprising 108 base pairs (bp), an open reading frame of 495 bp encoding a polypeptide of 164 amino acids with an estimated molecular mass of 17.3 kDa, and a 3′ UTR of 281 bp including a significant poly(A) plus tail sequence. The predicted amino acid sequence of PcCypA shared high identity with CypA in other organisms. PcCypA transcripts were detected in the hepatopancreas, gill, heart, muscle, testis, and ovary of crayfish, with the highest expression levels in the heart. Western blot analysis found one 17-kDa band in all of the tissues examined, except for the ovary. Molecular identification and expression analysis of PcCypA will facilitate further studies of the immune defense mechanisms in red swamp crayfish, and provide new insights into freshwater invertebrate immunology.

Keywords: Cyclophilin A; *Procambarus clarkii*; Real-time PCR; Western blot; Open reading frame

1. Introduction

Cyclophilins (Cyps) comprise a family of proteins found in vertebrates and other organisms that bind to cyclosporine, which is an immunosuppressant used to inhibit rejection after internal organ transplantation [1]. Diverse Cyps have been found in organisms ranging from bacteria to humans [2-6], including five classic Cyp isoforms in mammals (CypA, B, C, D, and cyclophilin 40), with sizes ranging from 18–40 kDa [7-9]. These proteins contain a single highly-conserved peptidyl-prolyl cis-trans

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