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Short communication

Channel catfish, *Ictalurus punctatus*, cyclophilin A and B cDNA characterization and expression analysis

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Abstract

The preliminary observation of up-regulation of cyclophilin transcripts during *Edwardsiella ictaluri* infection prompted us to speculate on the potential importance of cyclophilins in the early stage of infection. To provide a framework for answering these questions, two cyclophilin cDNA of channel catfish, *Ictalurus punctatus*, were identified, sequenced and characterized. The complete nucleotide sequences of cyclophilin A and cyclophilin B cDNA consisted of 1170 and 996 bases, respectively. Analyses of the sequences revealed each had one open reading frame potentially encoding 164 amino acids with calculated molecular mass of 17,450 Da and 216 amino acids with calculated molecular mass of 23,852 Da for cyclophilin A and cyclophilin B, respectively. The degrees of conservation of channel catfish cyclophilin A and cyclophilin B amino acid sequences to counterparts of other species ranged from 74 to 84% and 80 to 92%, respectively. Both cyclophilin A and cyclophilin B transcripts were constitutively expressed in all tissues of channel catfish examined in this study. These results provide valuable information not only for further exploring the roles of cyclophilins in fish immune responses to infection, but also for production of polyclonal/monoclonal antibodies for channel catfish cyclophilins.
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Keywords: Cyclophilin; Peptidyl-prolyl isomerase; Channel catfish; *Ictalurus punctatus*

1. Introduction

Cyclophilin A (CyPA) and cyclophilin B (CyPB) are intracellular members of the highly conserved immunophilin family and are ubiquitous (Bukrinsky, 2002; Galat, 1999; Ivery, 2000; Pemberton, 2006; Maruyama and Furuani, 2000; Wang and Heitman, 2005; He et al., 2004). Both proteins play many important roles in physiological and pathological processes. In physiological conditions, both proteins possess peptidyl-prolyl *cis–trans* isomerase activity that mediates protein folding as well as multidomain protein assembly, followed by serving as signal molecules (Wang and Heitman, 2005; Brazin et al., 2002; Min et al., 2005). In addition, Bukrinsky and other groups demonstrated that CyPA and CyPB use CD147, a widely expressed membrane protein, as a signal receptor resulting in chemotaxis and adhesion to the extracellular matrix, respectively (Allain et al., 2002; Yurchenko et al., 2001, 2002, 2005, 2006). CyPA binds strongly to the immunosuppressive agent, cyclosporine A, to form a complex that subsequently blocks T cell activation (Schmid, 2001; Heitman et al., 1992) via Itk tyrosine kinase (Brazin et al., 2002; Colgan et al., 2004). Another study demonstrated that CyPA interacts with peroxiredoxins and subsequently activates their peroxidase activity (Lee et al., 2001). A study has shown that CyPB and interferon regulatory factor-3 interaction

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resulted in suppression of phosphorylation of interferon regulatory factor-3 and thereby inhibits interferon-β induction (Obata et al., 2005).

On the other hand, cyclophilins have been implicated in microbial pathogenesis. Interactions of CyPA with various human immunodeficiency virus-1 proteins are required for establishment of infection in human cells (e.g. Luban, 2007; Billich et al., 1995; Zander et al., 2003). CyPB interacts with the hepatitis C virus RNA polymerase NS5B and then promotes the replication of the viral genome (Watashi et al., 2005). In the course of studying *Edwardsiella ictaluri* pathogenesis, up-regulation of cyclophilin expressed sequence tags (EST) during *Ed. ictaluri* infection in channel catfish (CC) ovary cell (ATCC CRL-2772) line was observed (Yeh and Klesius, unpublished observation). This preliminary observation prompted us to speculate that these cyclophilins may play a critical role in the early stage of *Ed. ictaluri* infection. In order to provide a framework for answering these questions, CC CyPA and CyPB were isolated, characterized and expressed. Both cyclophilins were constitutively expressed in all tissues analyzed.

2. Materials and methods

2.1. Animals

Channel catfish (NWAC 103 strain, 20–25 g) were maintained at the Aquatic Animal Health Research Unit, Agricultural Research Service, United States Department of Agriculture, and were acclimated for 2 weeks at 28 °C before use in experiments (Jenkins and Klesius, 1998). All fish were clinically healthy. The protocol for animal usage in experiments was approved by the Institutional Animal Care and Use Committee, Aquatic Animal Health Research Unit, Agricultural Research Service, United States Department of Agriculture.

2.2. Tissue samples

Fish were euthanized by immersion in tricaine methanesulfonate (MS-222) according to the Guidelines for the Use of Fishes in Research (Nickum et al., 2004). Gills, skin, spleen, hepatopancreas, intestine and head kidneys were aseptically excised.

2.3. RNA isolation and construction of CC rapid amplification of cDNA ends (RACE)

Total RNA from the excised tissues was extracted by using a Tri reagent kit (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturer's protocol. The quality and quantity of total RNA was determined by an Agilent Bioanalyzer using RNA 1200 chips (Agilent Technologies, Santa Clara, CA). Both 16S and 28S RNAs were clearly identified.

For RACE library construction, a GeneRacer kit (Invitrogen Corp., Carlsbad, CA) was used according to the manufacturer's protocol. Both 5′- and 3′-RACE cDNAs were amplified by PCR. The primers synthesized by Sigma-Genosys (The Woodlands, TX) for PCR amplification are listed in Table 1. The PCR products were purified by agarose gel electrophoresis and ligated into a pCR4-TOPO TA vector (Invitrogen Corp.). The ligated plasmids were transformed into TOP10 *E. coli* by heat-shock. After enrichment in the S.O.C. medium, cells were streaked on LB plates containing 50 μg/ml of ampicillin and incubated at 35 °C overnight. Colonies were randomly picked and cultivated in WU medium for sequencing.

Table 1
Oligonucleotides used in this study

Primer	Sequence	Tm (°C)
GeneRacer 5′Primer (Invitrogen)	5′-CGACTGGAGCACGAGGACACTGA-3′	74
GeneRacer 3′Primer (Invitrogen)	5′-GCTGTCAACGATACGCTACGTAACG-3′	78
β-Actin F	5′-GACTTCGAGCAGGAGATGGG-3′	72
β-Actin R	5′-AACCTCTCATTGCCAATGGTG-3′	69
(1) Cyclophilin A[a]		
CPA38F	5′-GCAGCGGCAAGTGCTCTGCCAAGATA-3′	78
CPA42R	5′-GCTGCTGCTTCCTCTGCCTTCGACAA-3′	77
CPA207R	5′-ACCCTGGCACATGAAACCTGGGATGA-3′	77
(2) Cyclophilin B[a]		
CPB116F	5′-TCATGATCCAAGGAGGCGACTTCACCA-3′	77
CPB366F	5′-AGGCACAAAAACGGACGGCAGGGATA-3′	77
CPB299R	5′-CATGGCGTCTGCACGGTGGTAATGAA-3′	77
CPB385R	5′-TGCCGTCCGTTTTTGTGCCTTCAATC-3′	77

[a] Sequences used for cyclophilin A and B primer design were from DQ086168 and DQ086177, respectively.