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Peroxisome proliferator-activated receptor alpha1 in yellow catfish *Pelteobagrus fulvidraco*: Molecular characterization, mRNA tissue expression and transcriptional regulation by insulin *in vivo* and *in vitro*

Jia-Lang Zheng [a,b,c,1], Mei-Qin Zhuo [b,c,1], Zhi Luo [b,c,*], Yu-Feng Song [b,c], Ya-Xiong Pan [b,c], Chao Huang [b,c], Wei Hu [b,c], Qi-Liang Chen [b,c]

[a] National Engineering Research Center of Marine Facilities Aquaculture, Zhejiang Ocean University, Zhoushan 316022, China
[b] Key Laboratory of Freshwater Animal Breeding, Ministry of Agriculture of P.R.C., Fishery College, Huazhong Agricultural University, Wuhan 430070, China
[c] Freshwater Aquaculture Collaborative Innovative Centre of Hubei Province, Wuhan 430070, China

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ABSTRACT

Peroxisome proliferator-activated receptor alpha1 (PPARα1) cDNA was isolated from liver of yellow catfish *Pelteobagrus fulvidraco* by RT-PCR and RACE. Its molecular characterization, tissue expression and transcriptional regulation by insulin *in vitro* and *in vivo* were determined. PPARα1 mRNA covered 1879 bp, with an open reading frame (ORF) of 1410 bp encoding 469 amino acid residues, a 5′-untranslated region (UTR) of 49 bp, and a 3′-UTR of 421 bp. PPARα1 consisted of 4 domains, the A/B domain, DNA-binding domain (DBD), D domain, and ligand-binding domain (LBD). The predicted tertiary structure of yellow catfish PPARα1 showed an increased size of the main cavity that was made up of side chains from helices 3, 5, 10, 11, and 12. Changes of PPARα1 structure might affect binding of mammalian PPARα1-specific ligand and cofactor in yellow catfish and may endow yellow catfish PPARα1 with new ligand-independent or -dependent transactivation activity. PPARα1 was differentially expressed in various tissues during development. Furthermore, intraperitoneal injection *in vivo* and incubation *in vitro* of insulin reduced the mRNA expression of PPARα1 in the liver and hepatocytes of yellow catfish. Based on the observation above, the present study provides evidence that PPARα1 is differentially expressed within and among tissues during three developmental stages and also regulated by insulin both *in vivo* and *in vitro*, which warrants further investigation of PPARα1 physiological function in fish.

1. Introduction

Peroxisome proliferator-activated receptor alpha (PPARα), as its isotypes β and γ, is a member of the nuclear hormone receptor superfamily. PPARα has been shown to take part in diverse physiological processes, including maintenance of lipid homeostasis *via* regulation of the expression of genes and enzymes involved in lipid metabolism (Kono et al., 2009; Cho et al., 2012). Like other nuclear receptors, PPARα is composed of at least four domains with distinct functions. The A/B domain, located in the least conserved N-terminal region, is responsible for ligand-independent transactivation activity, comprising the activation function 1 (AF-1) element (Gelman et al., 1999a). DNA-binding domain (DBD) is composed of two zinc finger structures that bind specifically to PPAR response elements (PPREs) in the regulatory region of PPAR target genes. The D domain, a variable hinge region, followed by the large ligand and cofactor binding domain (LBD), allows conformational changes of the protein. LBD activity relies on the interaction of cofactors with a conserved AF-2 motif located in the C-terminal part of the LBD (Nolte et al., 1998; Michalik and Wahli, 2007). In fish, PPARα cDNA sequences have been cloned and two PPARα transcripts, named as PPARα1 and PPARα2, have been found in several fish species, such as zebrafish, medaka, tilapia and tetradon (see Fig. 2 for Ensemble number). High PPARα mRNA expression has been reported in tissues such as heart and muscle with high β-oxidation capacity (Batista-Pinto et al., 2005; Leaver et al., 2005; Tsai et al., 2008; Zheng et al., 2013). However, very limited studies have been conducted to investigate tissue- and/or developmental stage-specific expression of PPARα (Ibabe et al., 2005).

Insulin is a peptide hormone that plays a fundamental role in the regulation of somatic growth and lipid metabolism in all vertebrates (Caruso and Sheridan, 2011). Insulin could stimulate PPARα phosphorylation and enhance its transcriptional activity (Shalev et al., 1996).

Abbreviations: BW, body weight; DBD, DNA-binding domain; FBS, fetal bovine serum; LBD, ligand-binding domain; NJ, neighbor-joining; ORF, open reading frame; PPAR, peroxisome proliferator-activated receptor; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription polymerase chain reaction; SEM, standard errors of means; UTR, untranslated region.

* Corresponding author. Tel.: +86 27 8728 2113; fax: +86 27 8728 2114.
E-mail addresses: luozhi99@mail.hzau.edu.cn, luozhi99@aliyun.com (Z. Luo).

[1] These authors contributed equally to the work.

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Furthermore, PPARα activators improved insulin sensitivity in rat (Guerre-Millo et al., 2000). Thus, it is reasonable to speculate that insulin exerts its physiological role in part mediated by PPARα.

Yellow catfish (*Pelteobagrus fulvidraco*) is an omnivorous, freshwater species of fish with increasing interest in Chinese inland aquaculture. At present, intensive aquaculture practices for yellow catfish often lead to excess lipid deposition in the fish species, which has adversely influenced growth performance and health. Recently, we cloned the partial cDNA sequence of PPARα and investigated its mRNA tissue expression profiles for yellow catfish (Zheng et al., 2013). As a continuation of our studies involved in the structure and functions of the gene, the present study cloned the full cDNA sequences of PPARα1 in yellow catfish by RT-PCR and RACE, and their tissue-specific and developmental expressions were determined. The transcriptional regulation of PPARα1 by insulin *in vivo* and *in vitro* was also investigated. The present study could provide new insights to the physiological function of PPARα in fish.

2. Materials and methods

Here, two experiments were conducted. The first experiment was involved in PPARα1 cloning and mRNA tissue expression during the developmental stage. The second experiment was designed to evaluate the transcriptional regulation of PPARα1 by insulin *in vivo* and *in vitro*. We assured that the experiments performed on animals and cells followed the ethical guidelines of Huazhong Agricultural University.

2.1. Experiment 1: PPARα1 cDNA cloning and tissue expression patterns during the three developmental stages

2.1.1. Fish and tissue sampling

Two groups of yellow catfish were used. The first group of three fish (mean weight: 25 g) was obtained from a local commercial farm, and used for the gene cloning study. The second group of yellow catfish came from the same farm and was used in the developmental study. Three stages were chosen: larvae (body weight 0.15 ± 0.03 g, 1-month old), juvenile (body weight 4.5 ± 0.5 g, 2-month old), and adult (body weight 20.6 ± 1.8 g, 4-month old). The larval yellow catfish were hatched on June 2013 and raised in indoor tanks. They were provided commercial diets (lipid and protein contents, 9.4% and 42.6% of dry matter basis, respectively) to satiation twice a day and continuous aeration to maintain the dissolved oxygen level near saturation. Dechlorinated tap water in each tank was replenished 100% twice daily, before feeding. Care was taken to ensure that no uneaten feed remained in the tanks during feeding. Fecal matter was quickly removed during the experiment. The experiment was conducted at ambient temperature and subjected to a natural photoperiod (approximately 14 h light/10 h dark). Water quality parameters were monitored in the morning twice a week. Water temperature ranged from 28 °C to 33 °C; dissolved oxygen 5.8 mg/l and total ammonia-nitrogen 0.04–0.05 mg/l.

At the end of the first, second and fourth months, fish were starved for 24 h before sampling. Fish were euthanized (MS-222 at 10 mg/l). Liver, heart, muscle, intestine, brain, visceral adipose, spleen, kidney, and gill were removed on the ice and rapidly frozen in liquid nitrogen for subsequent analysis of mRNA expression levels of PPARα1.

2.1.2. RNA isolation, reverse transcription, and fragment amplification

Frozen tissues were powdered in a liquid nitrogen-chilled mortar and pestle. Total RNA was extracted from liver using RNAiso plus (TaKaRa, Tokyo, Japan) based on the acid guanidinium thiocyanate–phenol–chloroform extraction method. Contaminating genomic DNA was eliminated using RNase-free Dnase. Two micrograms of total RNA was used for reverse transcription with TaKaRa PrimeScript™ RT-PCR Kit, following the protocol of the manufacturer. Two microliters of the cDNA obtained were used as a template for PCR. Amplifications by PCR were carried out (30 amplification cycles at 95 °C for 1 min, 57 °C for 1 min and 72 °C for 1 min) using oligonucleotide primers previously deduced from alignment using Clustal-W software (Infobiogen, Larkin et al., 2007) of corresponding sequences available in library from different fish and mammalian species (Table 1). The resulting target fragments were purified using the Agarose Gel DNA Fragment Recovery Kit Ver.2.0 (TaKaRa, Japan), subcloned using the PMD19-T Easy Vector System (Promega, USA). The plasmid was used for transformation of JM109 Competent Cells and Clones with inserts were sequenced.

2.1.3. Full-length PPARα1 cDNA amplification

Full-length PPARα1 cDNA of yellow catfish was obtained by the procedures of rapid amplification of cDNA ends (RACE) method with the gene-specific primers (Table 1). 3′RACE was performed using a 3′-Full RACE Core Set, according to the manufacturer's instructions. Firstly, reverse transcription of 2 μg of total RNA was performed with Oligo (dT) 16AP as a primer, which contained an anchor sequence. The resulting first strand cDNA was then diluted to 1:10 with ddH_2O and used as a template for PCR. The expected fragment was obtained with two gene-specific forward primers and two reverse primers by the nested PCR. 5′RACE was carried out following a protocol described by Zhang and Frohman (2000). Briefly, reverse transcription was conducted according to 3′RACE. After RNase H treatment, an Oligo (dA) tail at the 5′ end was added using terminal deoxynucleotidyl transferase. The resulting product was diluted to 1:10 with ddH_2O, and used as a template for PCR. The expected fragment was obtained with two gene-specific forward primers and two reverse primers by the nested PCR. PCR conditions for the RACE reactions were as follows: 30 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min, with a final extension step of 5 min at 72 °C. The 3′ and 5′ target fragments were purified, subcloned and sequenced according to the methods mentioned above. The assembled sequence from the core fragment, 3′ end and 5′ end sequences was further confirmed by amplifying the complete sequence using a pair of primers as shown in Table 1.

2.1.4. Sequence analysis

The core fragment, 3′ end and 5′ end sequences were assembled using SeqMan II software in DNASTAR PACKAGE to obtain full-length cDNA. The sequence was edited and analyzed using the program EDITSEQ of DNASTAR package to search for the open reading frame (ORF) and then translated into an amino acid sequence using standard genetic codes. The nucleotide sequences were compared with DNA sequences present in the GenBank database using BLAST network service at the NCBI (http://blast.ncbi.nlm.nih.gov/). Sequence alignments and percentage of amino acid conservation were assessed with the Clustal-W multiple alignment algorithm. The program PROSHAPE (http://csb.stanford.edu/koehl/ProShape/) was used to identify and characterize the presence of a groove in the TE structure. The phylogenetic tree was constructed with MEGA 5.0 (Tamura et al., 2011) by the neighbor-joining (NJ) method based on the JTT+G model (Jones et al., 1992), and the best-fit model of sequence evolution was obtained by ML model selection. Secondary structures of PPARα1 were performed using PredictProtein (http://www.predictprotein.org/). Three-dimensional structures of PPARα1 are obtained using SWISS-MODEL (Arnold et al., 2006). The ligand binding pocket search is performed with the program Pocket-finder (Hendlich et al., 1997). The figure was made with Chimera 1.8.

2.2. Experiment 2: transcriptional regulation of PPARα1 by insulin in vivo and in vitro

Bovine insulin was purchased from Sigma (Sigma, USA). Yellow catfish were cultured indoors as described above. The experiment was divided into two parts. In part 1, fish were intraperitoneally injected with insulin. In part 2, primary hepatocytes of yellow catfish were incubated with insulin. Both experiments continued for 48 h.