Biochemical and Biophysical Research Communications 481 (2016) 38–45

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Cloning and expressions of peroxisome proliferator activated receptor alpha1 and alpha2 (*PPARα1* and *PPARα2*) in loach (*Misgurnus anguillicaudatus*) and in response to different dietary fatty acids

Xiao Liang, Jian Gao*, Dapeng Li, Xiaojuan Cao

College of Fisheries, Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education/Key Lab of Freshwater Animal Breeding, Ministry of Agriculture, Huazhong Agricultural University, Wuhan 430070, China

ARTICLE INFO

Article history:
Received 3 November 2016
Accepted 5 November 2016
Available online 7 November 2016

Keywords:
Misgurnus anguillicaudatus
PPARα1 and *PPARα2*
Gene cloning
Spatio-temporal expressions
Dietary fatty acids

ABSTRACT

Peroxisome proliferator activated receptor alpha1 and alpha2 (*PPARα1* and *PPARα2*) were investigated in loach (*Misgurnus anguillicaudatus*) by RACE (rapid amplification of cDNA ends) and qPCR (real-time quantitative PCR) for the first time. The cDNA sequences of *PPARα1* and *PPARα2* were 2042bp and 2407bp, respectively encoding 467 and 465 amino acids. Sequence alignments of deduced amino acids showed significant homology between the two subtypes of *PPARα*, indicating 70% identity. The two genes revealed sensible changes in transcriptions during early life stages of the loach, and the highest transcriptions of the two genes both appeared at some day after hatching. *PPARα1* predominantly expressed in liver, while *PPARα2* markedly expressed in heart. The expression regulation of *PPARα1* and *PPARα2* in response to dietary fatty acids was determined in livers of loaches fed with diets containing fish oil (FO group) and soybean oil (SO group) for 75 days. The expression level of *PPARα1* in FO group was significantly higher than those in SO group ($P < 0.01$), while the expression level of *PPARα2* in FO group was also significantly higher than those in SO group ($P < 0.05$). There was no significant difference in the expression level between *PPARα1* and *PPARα2* in SO group, whereas significant difference in FO group. These indicated that lipid resources could regulate the expressions of these two genes in the loach. Our results will provide opportunities to better understand the functional characterization of *PPARα1* and *PPARα2* in further studies.

1. Introduction

Peroxisome proliferator activated receptor alpha (*PPARα*), an important ligand-modulated transcription factor, belongs to the members of superfamily of nuclear receptors [1]. Present studies have demonstrated that *PPARα* is vital regulator of physiological processes, like lipid, glucose metabolism and inflammatory responses [2,3]. A heterodimer of *PPARα* and retinoic acid X receptor (*RXR*) regulates the expression of downstream genes through binding the peroxisome proliferator response elements (*PPREs*), which located in the promoter regions of target genes [3,4]. As a key transcriptional factor, *PPARα* has been found in diverse species [1,5], while two subtypes of *PPARα* (namely *PPARα1* and *PPARα2*) have been reported in few fishes, such as zebrafish (*Danio rerio*) [6], turbot (*Scophthalmus maximus*) [7], yellow catfish (*Pelteobagrus fulvidraco*) [8] and yellow croaker (*Larimichthys crocea*) [9]. Moreover, there are few studies of spatio-temporal transcriptions of the two subtypes of *PPARα* [10].

As an important nutritional resource, lipids in diets provide energy and essential fatty acids (EFAs), and dietary EFAs are necessarily required for normal growth and survival [11]. Fish oil (FO) and vegetable oils (for instance, soybean oil (SO)) are important lipid resources. FO is rich in n-3 LC-PUFA, such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). SO has low concentration of n-3 LC-PUFA, but is rich in C18 fatty acids like linoleic acid (LA, 18:2n-6) and linolenic acid (LNA, 18:3n-3). In addition, not only can dietary fatty acids affect the capability of desaturase and elongase to produce the long-chain polyunsaturated fatty acids (LC-PUFAs) from C18 fatty acids [12,13], but also act as available endogenous ligands of *PPARα* [14]. However, the effects of different lipid resources on *PPARα* subtypes in

* Corresponding author. No. 1, Shizishan Street, Hongshan District, Wuhan, Hubei Province, 430070, China.
E-mail address: gaojian@mail.hzau.edu.cn (J. Gao).

http://dx.doi.org/10.1016/j.bbrc.2016.11.022
0006-291X/

fish were still not clear.

Dojo loach (*Misgurnus anguillicaudatus*), belonging to the family Cobitidae, is a widely distributed demersal Cobitidae fish in eastern Asia. The advantages, including high nutritional and medicinal value, made its high market value [15]. In this study, we firstly cloned two subtypes of *PPARα* (*PPARα1* and *PPARα2*) from the loach liver, and then their molecular characterizations and expression profiles in early life stages and different tissues were investigated. At last, the transcriptions of *PPARα1* and *PPARα2* regulated by dietary lipid sources were determined. This study could be foundation of exploring the functional characterization of *PPARα1* and *PPARα2* in further studies.

2. Materials and methods

2.1. Compliance with ethics guidelines

This study was conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Huazhong Agricultural University. All efforts were made to minimize suffering of the loaches.

2.2. Fish and sampling collections

The wild-adult loaches (body mass about 20 g) were collected from Ezhou City in China and kept at a stable temperature (26 ± 2 °C) in aquaria. All loaches were subjected by a ploidy analyser (Partec, Germany) to determine their ploidy levels. Diploid loaches were retained here. The livers from three loaches were used for cloning *PPARα1* and *PPARα2*. Two male and two female loaches were used for reproduction. Offsprings were collected at different early life stages, including unfertilized spermatozoa (US), unfertilized ovum (UO), fertilized egg (F), 2 cell (2C), 8 cell (8C), 32 cell (32C), blastula (B), gastrula (G), neurula (N), tail-bud forming (organ formation stage) (TB), heart-beating stage (HB), hatching (H), and second day after hatching (2nd), tenth day after hatching (10th), twentieth day after hatching (20th) and thirty fifth day after hatching (35th). The feeding regimes of the loach offsprings were based on the methods from our laboratory described by Gao et al. [16]. Samples of each early life stage were collected from 15 loaches and each five loaches presented as a biological repeat. Ten tissues including muscle, kidney, heart, spleen, liver, intestines, gill, brain, testis and ovary (n = 3) were collected from adult loaches. All samples were frozen at −70 °C prior to RNA extraction.

2.3. Dietary experiment and sampling

Loach juveniles (one-month-old) obtained by us were acclimated into six 50 L indoor tanks with flow-through fresh water system and the stocking density was 20 fish/tank. The feeding trial was supplied with a temperature at 24.1 ± 0.2 °C (mean ± SD) under a photoperiod of 12 h light/12 h dark. The average initial body weight of loach juveniles was 0.4 g. Loaches were fed with two diets containing FO (FO group) and SO (SO group) for 75 days and each treatment was set with triplicate. Feeding was done twice a day (08:00 and 20:00 h). Diets for loaches were made referring to the methods described in Gao et al. [16]. The formulation compositions of the experimental diets are presented in Table 1. The analysis of diets' proximate compositions followed the methods of AOAC [17]. After the feeding experiment, the growth performances of loaches were evaluated, meanwhile, livers of three loaches from each dietary treatment were collected and frozen at −70 °C prior to RNA extraction.

Table 1
Formulations and proximate compositions of the experimental diets.

Ingredients %	FO	SO
Defatted fish meal[a]	5	5
Soybean concentrate	33	33
Canola meal	20	20
Wheat meal	9	9
Starch	10	10
Dextrin	8	8
Carboxyl-methyl cellulose (CMC)	4	4
Fish oil[b]	7	
Soybean oil		7
Vitamin mix[c]	1	1
Mineral mix[d]	1	1
Calcium biphosphate	2	2
Proximate composition		
Crude lipid (% of dry mass)	7.1	7.0
Ash (% of dry mass)	9.7	9.4
Crude Protein (% of dry mass)	36.8	35.9
Moisture (%)	10.2	9.8

[a] Fishmeal had been skimmed by 100% diethyl ether.
[b] J. Oil Mills, Tokyo, Japan.
[c] Vitamin mixture (mg/kg diet): ß-carotene 128.4; Vitamin $D_3$12.9; Menadione $NaHSO_3 \cdot 3H_2O$ (K_3) 61.1; Thiamine-Nitrate (B_1) 66.7; Riboflavin (B_2) 256.6; Pyridoxine-HCl (B_6) 61.1; Cyanocobalamine (B_{12}) 0.1; d-Biotin 7.78; Inositol 5132.2; Niacine (Nicotic acid) 769.73; Ca Panthothenate 269.49; Folic acid 19.2; Choline choloride2098.30; ρ-Aminobenzoic acid 102.25.
[d] Mineral mixture (mg Kg^{-1} diet): $MgSO_4$ 5070; Na_2HPO_4 3230; K_2HPO_4 8870; Fe Citrate 1100; Ca Lactate12090; Al $(OH)_3$ 10; $ZnSO_4$ 130; $CuSO_4$ 4; $MnSO_4$ 30; Ca $(IO_3)_2$ 10; $CoSO_4$ 40. FO, fish oil; SO, soybean oil.

2.4. Cloning of PPARα1 and PPARα2 cDNAs in loach

Total RNA was isolated from the liver (40–60 mg) using RNA isoPlus (TaKaRa, Japan). Quantities and qualities of isolated RNAs were ascertained by electrophoresis and spectrophotometry (Nanodrop 2000, Thermo Scientific, USA). Then samples with an absorption ratio (1.8–2.0) were stored for cDNA synthesis. Reverse transcription was conducted with PrimeScript®RT reagent Kit with gDNA Eraser (TaKaRa, Japan), and the 3' and 5' ends of cDNAs were cloned using the RACE-PCR with the SMART RACE Kit (Clontech, USA). The universal amplified primers of loach *PPARα1* and *PPARα2* were respectively designed on distinct conserved regions from multiple alignments of various fishes including zebrafish (*Danio rerio*), turbot (*Scophthalmus maximus*) and medaka (*Oryzias latipes*). The primers to amplify the partial sequences are listed in Table 2. Parameters for PCR were as follows: 30 cycles of 30 s at 95 °C, 30 s at 57 °C and 1 min at 72 °C, an initial 94 °C denaturation for 5 min and a 72 °C extension for 8 min.

Primers designed for end amplifications are listed in Table 2, and the RACE was conducted with the manufacturer's instructions (Clontech, USA). The annealing temperature was 62 °C for extending sequences. The PCR products described above were all purified from 2.0% agarose by SanPrep Column DNA Gel Extraction Kit (Sangon, China) and loaded into the pMD 19-T cloning vector (TaKaRa, Japan). Then the positive transformants were selected and sequenced (Sangon, China).

2.5. Sequence and phylogenetic analysis

The fragments of *PPARα1* and *PPARα2* were assembled by BioEdit, and the putative amino acid sequences were predicted using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Other vertebrate PPARα amino acid sequences for alignments and constructing phylogenetic trees were obtained from the Protein databases (NCBI) and the identities of these sequences were blasted by Blastp (http://blast.ncbi.nlm.nih.gov/). The method of neighbor-joining (NJ) (bootstrap method: 1000 replications) was used to