Genomics 101 (2013) 195–203

Contents lists available at SciVerse ScienceDirect

Genomics

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

Molecular characterization, tissue distribution and kinetic analysis of carnitine palmitoyltransferase I in juvenile yellow catfish *Pelteobagrus fulvidraco*

Jia-Lang Zheng, Zhi Luo *, Qing-Ling Zhu, Qi-Liang Chen, Yuan Gong

Fishery College, Huazhong Agricultural University, Wuhan 430070, China

ARTICLE INFO

Article history:
Received 10 August 2012
Accepted 2 December 2012
Available online 10 December 2012

Keywords:
Pelteobagrus fulvidraco
Carnitine palmitoyltransferase I
Isoforms
mRNA expression
Kinetic characteristics

ABSTRACT

Up to date, only limited information is available on genetically and functionally different isoforms of CPT I enzyme in fish. In the study, molecular characterization and their tissue expression profile of three CPT Iα isoforms (CPT Iα1a, CPT Iα1b and CPT Iα2a) and a CPT Iβ isoform from yellow catfish *Pelteobagrus fulvidraco* is determined. The activities and kinetic features of CPT I from several tissues have also been analyzed. The four CPT I isoforms in yellow catfish present distinct differences in amino acid sequences and structure. They are widely expressed in liver, heart, white muscle, spleen, intestine and mesenteric adipose tissue of yellow catfish at the mRNA level, but with the varying levels. CPT I activity and kinetics show tissue-specific differences stemming from co-expression of different isoforms, indicating more complex pathways of lipid utilization in fish than in mammals, allowing for precise control of lipid oxidation in individual tissue.

1. Introduction

Carnitine palmitoyltransferases I and II (CPT I and CPT II), together with the acyl-carnitine translocase, mediate the transfer of acyl-groups into mitochondria [1], and play an important role in the regulation of mitochondrial β-oxidation in all vertebrates, including fish. CPT I (EC 2.3.1.21), located in outer membranes of mitochondria, catalyzes the carnitine-dependent esterification of palmitoyl-CoA to form palmitoylcarnitine [2,3]. CPT II, located on the inner mitochondrial membrane, catalyzes a second esterification, generating palmitoyl-CoA and carnitine inside the mitochondrial matrix [4]. In vertebrates, CPT I has a key function controlling the flux through β-oxidation, and is the main regulatory step of fatty acid oxidation [5]. Therefore, understanding of the mitochondrial CPT I protein in terms of its structure/function/regulatory properties has become a matter of intense biochemical and genetic interest.

In mammals, three CPT I isoforms encoded by distinct genes with various tissue distribution have been identified: a liver isoform (CPT Iα or L-CPT I) [6], a muscle isoform (CPT Iβ or M-CPT I) [7], and a brain isoform (CPT IC) [8]. Whereas CPT Iα is widely expressed in most tissues such as liver, spleen, intestine and heart, CPT Iβ is mainly expressed in muscle, adipose tissue, heart and testis [1]. CPT IC expression is restricted to the central nervous system [8]. At present, it is not clear whether these isoforms are conserved across vertebrates or how many additional isoforms exist in non-mammalian species, particularly in fish [9]. At present, several studies have suggested that putative presence of the α- and β-isoforms of CPT I is existent in rainbow trout [9–12] and gilthead seabream [13], and little information is available for other fish species. Also, there is little information available on their tissue expression, protein structure or kinetics [9].

Yellow catfish *Pelteobagrus fulvidraco*, an omnivorous freshwater fish, is widely distributed in Chinese inland aquaculture. The fish species have been considered to be a good model to study lipid metabolism. At present, the regulation of lipid metabolism at the gene level has been poorly studied in yellow catfish. In the present study, we describe the cloning, molecular characterization and the tissue expression profile of four CPT I isoforms, and investigate kinetic parameters of CPT I in various tissues of yellow catfish. We also assess the primary protein structure for changes in amino acids in areas that may influence malony-CoA sensitivity and CPT kinetics between tissues and species.

2. Materials and methods

2.1. Animals

Eighty juvenile yellow catfish (initial body weight: 50 ± 5 g) were obtained from a local farmer. They were maintained in four, 300–l circular fiberglass tanks for 2-wk acclimatization, with 20 fish for each tank. During the acclimatization period, they were provided commercial Haid® diet (crude lipid and protein contents: 9.8% and 33.6% on a 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 no uneaten feed remained in the tanks during feeding. Fecal matter was also quickly removed during the experiment.

* Corresponding author. Fax: +86 27 8728 2114.
E-mail address: luozhi99@yahoo.com.cn (Z. Luo).

0888-7543/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygeno.2012.12.002

The experiment was conducted at ambient temperature and subjected to natural photoperiod (approximately 10 h light/14 h dark). Water quality parameters were monitored twice a week in the morning. Water temperature ranged from 18.2 °C to 20.4 °C; dissolved oxygen 5.8 mg l^{-1}; total ammonia–nitrogen 0.032–0.056 mg l^{-1}.

At the end of 2-wk acclimatization, approximately 24 h after the last feeding, fish were euthanized (MS-222 at 10 mg l^{-1}). Liver, heart, white muscle, spleen, intestine and mesenteric adipose were removed on the ice, rapidly frozen in liquid nitrogen (not exceeding one week) for subsequent analysis. We assured that the experiments performed on animals followed the ethical guidelines of Huazhong Agricultural University for the care and use of laboratory animals.

2.2. Cloning of CPT I cDNA

2.2.1. Design of primer sequences

Liver isoform sequences (L-CPT I) from zebrafish (GenBank accession no. NM_001044854), orange-spotted grouper (GenBank accession no. HM037343) and rainbow trout (GenBank accession no. GU592679), muscle isoform (M-CPT I) sequences from zebrafish (GenBank accession no. NM_001005940), rainbow trout (GenBank accession no. AJ606076) and gilthead seabream (GenBank accession no. DQ866821) were compared by using Clustal-W multiple alignment algorithm. Different degenerated primers were chosen and designed in the most conserved regions of the CPT I sequence for amplification of a partial CPT I cDNA sequence using total RNA from different tissues. 24 primers as the forward gene-specific primer CPT 5′F were designed according to degeneracy of amino acids including initiation codon region, in order to amplify 5′cDNA extremities combined with reverse gene-specific primer CPT 5′R designed from a partial CPT I cDNA sequence (Table 1). The sequences encoding the carboxy-terminus of CPT I was obtained through 3′-rapid amplification of cDNA ends PCR (3′-RACE).

2.2.2. RNA isolation and synthesis of cDNA

Frozen tissues were powdered in a liquid nitrogen-chilled mortar and pestle. Total RNA was extracted from the liver, white muscle, heart, brain, spleen, intestine and mesenteric adipose using TRIzol Reagent (Invitrogen, Carlsbad, USA) based on the acid guanidinium thiocyanate–phenol–chloroform extraction method. Two microgram of total RNA was used for reverse transcription with RevertAid™ Reverse Transcriptase (Fermentas, Burlington, Canada) and an oligo-dT primer. Two microliters of the cDNA obtained were used as template for PCR with the forward primer CPT 5′F and the reverse primer CPT 5′ R. PCR cycles were conducted at 95 °C for 3 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and a final cycle of 72 °C for 10 min. The resulting target fragments were purified using the Agarose Gel DNA Fragment Recovery Kit Ver. 2.0 (TaKaRa, Shiga, Japan), subcloned using the pGEM-T Easy Vector System (Promega, Nepean, Canada). The plasmid was used for transformation of JM109 Competent Cells (Promega, Nepean, Canada) and clones with inserts were sequenced (Sangon, Shanghai, China). The sequences encoding the carboxy-terminus of CPT I were obtained through 3′-RACE using 3′-Full RACE Core Set Ver. 2.0 (TaKaRa, Shiga, Japan) with the oligo-dT anchor primer provided by the kit and the gene-specific primer. Amplification was carried, using the reaction settings as follows: initial denaturation at 95 °C for 4 min, followed by 30 cycles of denaturing for 30 s at 94 °C, annealing for 30 s at 55 °C, extension for 2 min at 72 °C, and a final extension for 10 min at 72 °C. The final PCR product was purified, cloned, and sequenced as described above.

2.2.3. Sequence analysis

The sequence was edited, 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 phylogenetic tree was generated using neighbor-joining (NJ) methods based on CPT I amino acid sequences using the PAUP 4b software packages (http://paup.csit.fsu.edu/about.html). Putative transmembrane regions were predicted by TMpred (http://www.ch.embnet.org/software/TMPRED_form.html).

2.3. mRNA quantification by real-time PCR

Extraction of total RNA from fish tissues and first strand cDNA synthesis were performed as described above. The cDNA synthesis reactions were diluted to 200 μL in water. Q-PCR reactions (20 μL) were performed in 96-well plates in a Bio-Rad iQ5 (Germany) with GoTaq®qPCR Master (Promega, Nepean, Canada), containing 10 μL GoTaq®qPCR Master mix, 2 μL of cDNA, and 0.2 μM of each primer. The primer sequences used in the present analysis are given in Table 1. The PCR parameters were initial denaturation at 95 °C for 15 min, followed by 45 cycles at 95 °C for 15 s, 57 °C for 10 s (annealing temperature Tm + 2 °C for all primers pairs except for β-actin whose annealing temperature was Tm + 1 °C) and 72 °C for 30 s, and a final extension for 10 min at 72 °C. All reactions were performed in duplicates and each reaction was verified to contain

Table 1
Primers used for sequencing of CPT I (S) and real-time PCR analysis (Sq) from yellow catfish (fragments were noted in Fig. 1).

Gene	Fragments	Forward primer (5′-3′)	Reverse primer (5′-3′)	Step	Tm, °C	Size, bp
CPT Iα1b	S1	ATGGCAGAAGCTCATCAAGC	TCCCACCARTCRCTBACRTARTT	RT-PCR	55	713
	S2	TTTCAGAAGACCCTGGCTCCC	TGCAGSAGKGAYTTDGCRTA	RT-PCR	55	704
	S3	AGACAGGGGAAGAACAGACAAAGT	TACCGTCGTTCCACTAGTGATTT	3′RACE	55	1268
	Sq1	ATGACGGTTATGGAGTCGCTTAT	GTGTCACTTCAGGGCTTTCGT	Q-PCR	55	176
CPT Iα1a	S1	TGCTAYRACAGGTGGTTYGAYAA	TAACCRTCATCDGCMACHGGNCC	RT-PCR	55	821
	S2	AAYTAYGTVAGYGAYTGGTGGG	TCTTCAGTGTAGCCGAGCGTTAT	RT-PCR	55	806
	S3	ATGGCAGAAGCTCATCAAGC	TGAGCACGGTCCAGTTTCCT	RT-PCR	55	872
	S4	AATGGCTATGACTGGCAAGGG	TACCGTCGTTCCACTAGTGATTT	3′RACE	55	529
	S5	AAGTGCTGTCTGAGCCCTGGAG	CGCGGATCCTCCACTAGTGATTTCACTATAGG	3′RACE	55	444
	Sq2	AGCGATTGGTGGGAGGATTAT	TGAGCACGGTCCAGTTTCCT	Q-PCR	55	173
CPT Iα2a	S1	ATGGCAGAAGCTCATCAAGC	TCCCACCARTCRCTBACRTARTT	RT-PCR	55	713
	S2	TGCGTCCCCTGATGACAGAC	TGCAGSAGKGAYTTDGCRTA	RT-PCR	55	766
	S3	TCTGGAAGACGAGGAGCAAGG	TACCGTCGTTCCACTAGTGATTT	3′RACE	55	1112
	Sq3	GGAGGATTCGGACCTGTTGC	CGCTTTGTGGCTCTTCTTTCTTAT′	Q-PCR	55	198
CPT Iβ	S1	ATGGCAGAAGCGCATCAAGC	TCCCACCARTCRCTBACRTARTT	RT-PCR	55	713
	S2	AAAAGGACCAAGCCCCTAAACT	TGCAGSAGKGAYTTDGCRTA	RT-PCR	55	706
	S3	CCAAAGCACGACAGAAGCACT	TACCGTCGTTCCACTAGTGATTT	3′RACE	55	1363
	S4	GACACCACAGAGCCTCAGTCCG	CGCGGATCCTCCACTAGTGATTTCACTATAGG	3′RACE	55	1283
	Sq4	ACAAGAATGGTAAAATAGGGGTGA	TCCCTCCTCAGTGTAACCCAAG	Q-PCR	55	122
β-actin	Sq5	GCACAGTAAAGGCGTTGTGA	ACATCTGCTGGAAGGTGGAC	Q-PCR	56	136