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Identification of a soluble leptin receptor in crucian carp with different binding affinity to leptin-a and leptin-b

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ABSTRACT

Soluble leptin receptor (sLepR) is the main leptin-binding protein in plasma and contributes to activation of circulating leptin. In this study, we identified a sLepR in plasma of crucian carp (*Carassius carassius*) using a pull-down assay, and the interaction of sLepR with its ligand is confirmed by a cross-linking study. In addition, we found that leptin-a has higher affinity than leptin-b for sLepR. According to our knowledge, this is the first experimental report about the main ligand of sLepR in teleost.

1. Introduction

Leptin is a 167-amino acid peptide hormone, secreted primarily by adipose tissue. Its functions involved in feed intake, energy balance (Ahima and Flier, 2000), reproduction (Swain et al., 2004), immune function (Drazen et al., 2001) and bone growth (Steppan et al., 2000). In fish, several teleosts have duplicate leptin genes as a result of an early, large-scale genome duplication event (Taylor et al., 2003; Prokop et al., 2012). While leptin-a gene can be found in majority of fish, the leptin-b gene has been reported in zebrafish (Gorissen et al., 2009), Jian carp (Tang et al., 2013), orange-spotted grouper (Zhang et al., 2013) and salmonids (Angotzi et al., 2013).

The physiological actions of leptin depend on signaling through the leptin receptor (LepR), which is a member of the class I cytokine-receptor family. To date, at least six LepR isoforms have been identified in mammals. The long isoform of leptin Receptor (LepRb) has a complete cytoplasmic domain which activates the JAK/STAT signal transduction pathway. LepRa, c, d (in mice) and f (in rat) contain short cytoplasmic domains, and the soluble form (LepRe) contains only a extracellular domain binding to circulating leptin. In teleosts, the cDNA encoding the long form LepR was first isolated and characterized in marine medaka by Wong et al. (Wong et al., 2007). Since then, the long form of LepR genes have been identified from pufferfish (Kurokawa et al., 2008), Japanese medaka (Kurokawa and Murashita, 2009), rainbow trout (Murashita et al., 2008), zebrafish (Liu et al., 2010), grouper (Zhang et al., 2013), etc.

Soluble leptin receptor is generated by alternative splicing of LepR mRNA (LepRe) and/or ectodomain shedding of membrane-anchored leptin receptors. Soluble leptin receptor may delay the clearance of circulating leptin and thereby prolong its bioavailability (Zastrow et al., 2003). In addition, sLepR prevents leptin-binding to its membrane receptor, thus effectively inhibiting leptin-mediated STAT3 signaling (Zhang and Scarpace, 2009). In teleosts, it is first reported that the LepR gene in Atlantic salmon encodes four splicing variants which do not possess transmembrane domain (Ronnestad et al., 2010). In our previous study, we also identified three LepR splicing variants in crucian carp: cclpr-L (GenBank accession number *HQ993048*), cclpr-s1 (GenBank accession number *KT780702*) and cclpr-s2 (GenBank accession number *KT780702*), and the transmembrane domain of cclpr-s2 was absent (Cao et al., 2011). However, in teleosts, the detection of sLepR protein in plasma has only been reported in rainbow trout (Gong et al., 2013). The main aim of the present study is to identify the sLepR protein in plasma of crucian carp and analyze the binding affinity of leptin-a/b to sLepR.

2. Materials and methods

2.1. Animals

Crucian carps (average body weight: 250 ± 30 g) were purchased from a local supplier. After transportation to the experimental facilities, the fish were distributed in tanks supplied with aerated freshwater at 20 ± 1 °C and acclimatized to a daily ration of 1% of their estimated

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Table 1
Primer sequences used in PCR for leptin-a and leptin-b.

Primer sequence			
Gene	Purpose	Primer	5′–3′ sequence
Leptin-a	Partial cDNA	LepA F1	GAAGGTCAAAGGTCGTTC
		LepA R1	AACAGGCTCCAGGAGAG
Leptin-b	Partial cDNA	LepB F1	CCTGGTGGCTGTTAGCATCA
		LepB R1	TCAGAATAGAAACATCTCAGCC
	3′Race PCR	LepB F2	GATCCGAATCATGGCCCGAACT
		LepB F3	GGATGAGCACTTCCAGATGT
		LepB F4	ACCTCCAACTACCTGTACCTC

body weight for 1 week. Fish were anesthetized by 3-aminobenzoic acid ethyl ester (MS222, Sigma, USA) until the gill movements ceased. To isolate plasma, blood was collected from caudal vein in heparin-coated microtubes and spun for 5 min. Liver tissues were sampled and frozen immediately in liquid nitrogen.

2.2. Cloning of leptin-a and leptin-b cDNA for crucian carp

Total RNA was extracted from liver tissues using RNAiso Plus (Takara, Dalian, China) according to the manufacturer's instructions. The first-strand cDNA was prepared from total RNA using an oligo-dT primer with PrimeScript reverse transcriptase (Takara, Dalian, China). To amplify the cDNA fragment of crucian carp leptin-a and leptin-b, PCR primers were designed based on the previously reported sequences of *Carassius auratus*, *Cyprinus carpio*, *Ctenopharyngodon idella* and *Danio rerio* in GenBank database. The primers for 3′RACE were designed on the basis of the PCR result above and listed in Table 1. PCR products were purified using an agarose gel DNA purification kit (Sangon Biotech, Shanghai, China) and ligated into a pET 32a expression vector (Novagen, Wisconsin, USA), which produces a recombinant protein fused with histidine-tagged thioredoxin (Trx).

2.3. Generation of a polyclonal antibody against crucian carp LepR

A polyclonal rabbit antibody (anti-cclpr antibody) was raised against a synthetic peptide corresponding to positions 632–645 of crucian carp LepR. To determine the titers of anti-cclpr antibody, ELISA plates were coated with peptide$_{632-645}$ at 0.1 μg per well in 0.1 M carbonate buffer (pH 9.6). Serially diluted rabbit serum was added to wells of the coated plate and incubated for 1 h at room temperature. Goat anti-rabbit IgG conjugated with horseradish peroxidase (HRP) served as second antibodies. The antibody titer was defined as the highest serum dilution showing an absorbance 0.6 and achieved at a 240,000 dilution.

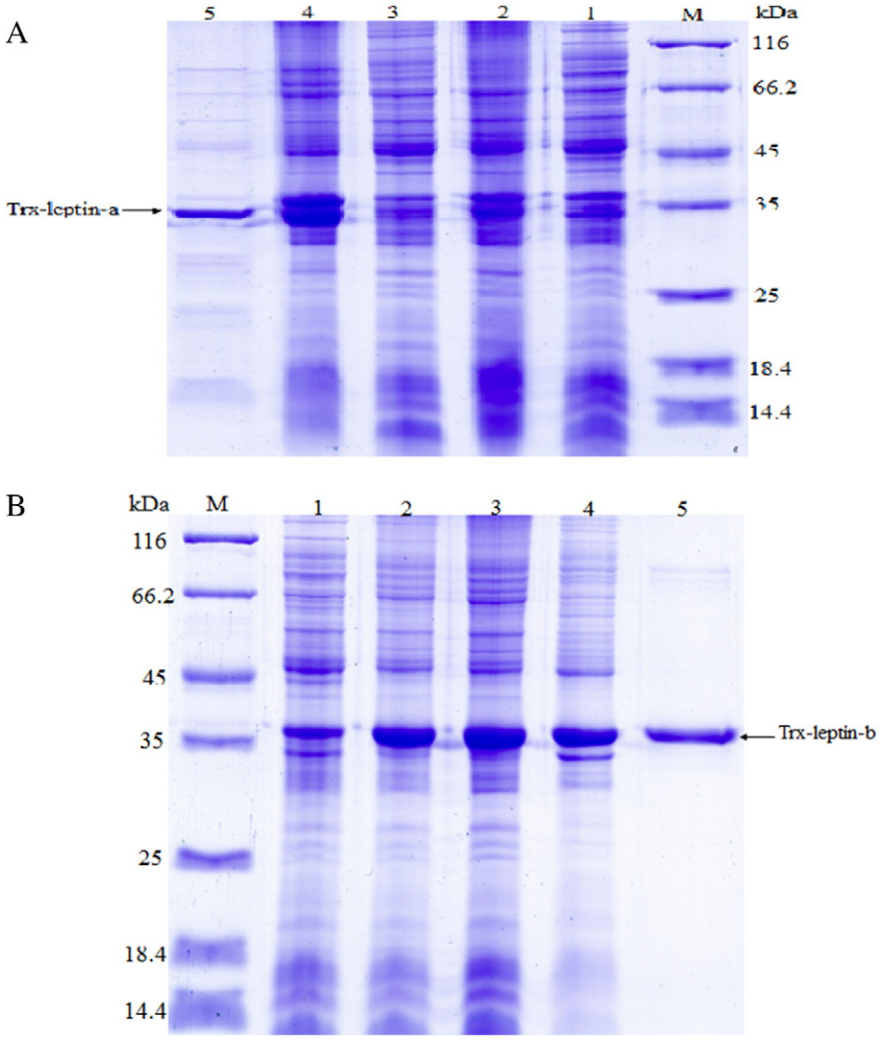

Fig. 1. SDS-PAGE analysis of the expression and purification of recombinant Trx-leptin-a (A) and Trx-leptin-b (B). Lane M: protein molecular weight markers (sizes given in kD). Lane 1: total crude proteins in uninduced cells. Lane 2: total crude proteins in induced cells. Lane 3: supernatant after IPTG induction. Lane 4: pellet after IPTG induction. Lane 5: the eluted Trx-tagged leptin.