Aquaculture 296 (2009) 110–116

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Molecular cloning and daily mRNA levels of prolactin and somatolactin in aquacultured Pacific bluefin tuna (*Thunnus orientalis*)

Kohsuke Adachi [a,c], Keitaro Kato [a,*], Masayoshi Yamamoto [a], Katsuya Ishimaru [a], Toru Kobayashi [b], Osamu Murata [a], Hidemi Kumai [a]

[a] Fisheries Laboratory, Kinki University, Shirahama 3153 Wakayama, Japan
[b] Laboratory of Aquatic Biology, Department of Fisheries, Faculty of Agriculture, Kinki University, Nara 631-8505, Japan
[c] Research and Education Faculty, Natural Sciences Cluster, Kochi University, Monobeotsu 200, Nankoku, Kochi 783-8502, Japan

ARTICLE INFO

Article history:
Received 7 August 2008
Received in revised form 14 July 2009
Accepted 22 July 2009

Keywords:
Daily rhythm
Pituitary hormone
Prolactin
Somatolactin
Teleost

ABSTRACT

We cloned the prolactin (PRL) and somatolactin (SL) genes of Pacific bluefin tuna (PBT: *Thunnus orientalis*) and investigated their daily mRNA expression patterns under aquacultured conditions. The PRL and SL cDNAs of PBT encode 211 and 231 aa prehormones which give rise to 187 and 207 aa mature peptides, respectively, and which belong to the same clades as those of other Perciformes. The highest gene expression levels of PRL and SL in PBT were found at 6 am under natural light conditions, which is about 3 times higher than the constitutive levels in both cases. Interestingly, scatter diagrams show a strictly correlated expression between PRL and SL ($r=0.82$; $p<0.01$) while no correlation was found between PRL or SL and the growth hormone (GH). These results suggest that PRL and SL play synchronized role(s) in daily physiological phases of PBT under aquacultured conditions.

1. Introduction

In recent years, the production of aquacultured Pacific bluefin tuna (PBT) (*Thunnus orientalis;* Perciformes–Scombridae) has increased rapidly and production has rocketed from 521 tons in 2001 to 3552 tons in 2006 (© FAO – Fisheries and Aquaculture Information and Statistics Service – 05/07/2008). However, basic knowledge of PBT, especially about their endocrine system, remains limited although they have fundamentally different physiological modes from other aquacultured fish such as their thermogenic system, metabolic rate, aerobic capacity, heart rate, gut clearance and cardiac physiology (Graham and Dickson, 2004). We previously reported that the expression of growth hormone (GH) transcripts reached a peak at 3–4 am at which time it was about 10 times higher than at any other time period (Adachi et al., 2008). Prolactin (PRL) and somatolactin (SL) belong to the GH/PRL/SL gene family in fish and presumably originated from a common ancestral molecule (Specker et al., 1985; Yasuda et al., 1986; Ono et al., 1990; Rand-Weaver et al., 1991). Since the pioneering studies in freshwater adaptation of killifish (*Fundulus heteroclitus*) (Pickford and Phillips, 1959), diverse physiological functions have been identified for PRL in fish, which include: water and electrolyte balance, metabolism, growth and development, reproduction, brain and behavior, and immunoregulation (Manzon, 2002; Power, 2005). SL was first identified in the Japanese flounder (*Paralichthys olivaceus*) (Ono et al., 1990). There is increasing evidence that in fish SL regulates energy homeostasis, the stress response, reproduction, calcium metabolism, acidosis, and pigmentation in teleosts (Kawauchi and Sower, 2006), among other functions, although no established category has been reported so far. These hormones show their own special functions, for example, PRL for osmoregulation (Manzon, 2002) and SL for pigment cell proliferation and lipolysis (Fukamachi et al., 2004, 2005), although their biological activities remain debatable. There have been several reports about nocturnal surges of PRL in mammals (Spiegel et al., 1994; Van-Cauter et al., 1998) and in fish there have been several reports about the night time secretion of PRL (Leatherland et al., 1974; Falcón et al., 2003). Zhu and Thomas (1998) investigated the effects of light and demonstrated that SL levels are significantly elevated in red drum (*Sciaenops ocellatus*) when no light is present. In that study, the plasma concentration of SL increased in the early part of the dark phase. These facts clearly indicate that expression of PRL and SL is controlled following the daily rhythm.

In this study, we cloned the cDNAs of the PRL and SL genes from PBT and measured their daily mRNA expression patterns to investigate potential correlations in expression of the GH/PRL/SL gene family.

2. Materials and methods

2.1. Molecular cloning of PRL and SL

2.1.1. RNA extraction and RT-PCR

Total RNA was extracted from the pituitary of PBT (*Thunnus orientalis*) (1 year old, 1400 g body weight and 433 mm total length)

* Corresponding author. Tel.: +81 739 42 2625; fax: +81 739 42 2634.
E-mail address: kei-kato@kindaisuiken.jp (K. Kato).

0044-8486/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2009.07.026

Table 1
Primers used in this study.

For molecular cloning and DNA sequencing	
prlgen F	ATGGCTCANAGRNAMASCRATGGAA
prlgen R	GCACATYCTNGGTYKCANTTTTGCNGCCC
slgen F	ATGCRCATGRTRASAGYMAT
slgen R	TGGGCRTCTTTCTTGAAGCAG
Tuna prl GSP 1	CCAATGGCTCAGACCATCTCATCAC
Tuna prl GSP 2	GGCCTATAGGAGGGAAATGAGAGT
Tuna sl GSP 1	CGCTATGATGATGCTCCCGACATGC
Tuna sl GSP 2	GCAGTGGGAATGGGACAAAC
Tuna prl seq F1	CAGCCACACACAGGAAATCA
Tuna prl seq F2	CCCTATCATGCCACCAGTTT
Tuna prl seq R1	CCAGCTGGATTCTCCAAGTC
Tuna prl seq R2	ACCAAATTCCCCCAAAATGT
Tuna sl seq F1	TGAATTCCTGGGGTTGGTAG
Tuna sl seq F2	TTGTATTCCTCGCTGGCTCT
Tuna sl seq R1	CGTCTCCGGTGAAAGTGTTT
Tuna sl seq R2	GGGCATCTTTCTTGAAGCAG
For realtime PCR of PBT	
Tuna prlrt F	AACTGCACTCCCTCAGCACA
Tuna prlrt R	CGAGCCAATGACAGCAGGTT
Tuna slrtF	TATGGGCTGTGTCGCTCTGG
Tuna slrtR	GCAGTGGGAATGGGACAAAC
Tuna b-actrtF	ACCCACACAGTGCCCATCTA
Tuna b-actrtR	TCACGCACGATTTCCCTCT

reared at the Ohshima station of the Fisheries Laboratory at Kinki University, Wakayama, Japan using the AGPC method (Chomczynski and Sacchi, 1987). First-strand cDNA was synthesized from 1 μg total RNA in 20 μl of a mixture of 50 units MMLV RTase (Takara Bio Inc, Otsu, Japan), 500 μM dNTP, 50 pmol oligo dT primer and 10 units RNase Inhibitor (Takara Bio Inc, Otsu, Japan). Reactions were carried out at 42 °C for 15 min. Sets of two mixed primers (prlgen F–R for PRL and slgen F–R for SL, shown in Table 1) were designed based on the published sequences of PRL and SL in other species. PCR was carried out in 50 μl of a mixture containing 1 unit High Fidelity Taq (Invitrogen, Carlsbad, CA), 1× reaction buffer, 200 μM dNTP, 2.0 mM $MgSO_4$ and 0.2 mM of a set of primers. Each reaction was carried out at 94 °C for 2 min, 30 cycles at 94 °C for 15 s, 55 °C for 15 s, and 68 °C for 1 min, followed by a final extension at 68 °C for 10 min using an ABI thermal cycler 9700.

2.1.2. Subcloning and DNA sequencing

The amplified products (about 650 bp for both PRL and SL) were separated on 1% agarose gels and were purified using a gel extraction kit (QIAGEN, Hilden, Germany) after which they were subcloned into the TOPO TA subcloning vector (Invitrogen, Carlsbad, CA). Both strands were sequenced using a dideoxyterminator cycle sequencing kit and a DNA sequencer Beckman coulter CQ 8000. Multiple clones were selected and sequenced.

2.1.3. 5′ and 3′ RACE for PRL and SL

Based on the partial sequences obtained, specific primers (tuna prl GSP 1 and 2 and tuna sl GSP 1 and 2 in Table 1) were designed, and then 5′ and 3′ RACE were performed using a Gene Racer Kit following the manufacturer's instructions. For the 5′ and 3′ RACE of PRL, fragments of approximately 250 and 1200 bp, respectively, were obtained, which were subcloned into the TOPO vector to be sequenced by the M13 forward and reverse priming reaction. For the 3′ RACE fragment, both strand sequences were determined using newly designed primers (tuna prl seq F1, 2 and R1, 2 in Table 1) based on the initial DNA sequencing. Multiple clones were selected and sequenced. Sequence data were compiled using GENETIX (SDC Software, Tokyo, Japan). For the 5′ and 3′ RACE of SL, the method described for PRL sequencing was carried out. The scheme is summarized in Fig. 1.

2.2. Analysis of the daily rhythm of PRL and SL mRNA expression

2.2.1. Animals

Juvenile PBT (42 days after hatching; 15.2 ± 4.2 g body weight and 144.3 ± 8.9 mm total length) were reared in large water tanks (1.5 × 3.6 × 3.6 m; 20,000 l) under a natural photoperiod at the Ohshima Station of the Fisheries Laboratory at Kinki University. The water temperature was maintained at 26.8–29.0 °C. Fish were fed to satiation at 1 h intervals during the day (from 5 am to 6 pm) with Japanese sand lance (*Ammodytes personatus*). Food was withheld for 24 h prior to 10 am on the first day of each experiment. To minimize stress during sampling, fish were collected using lures with six hooks

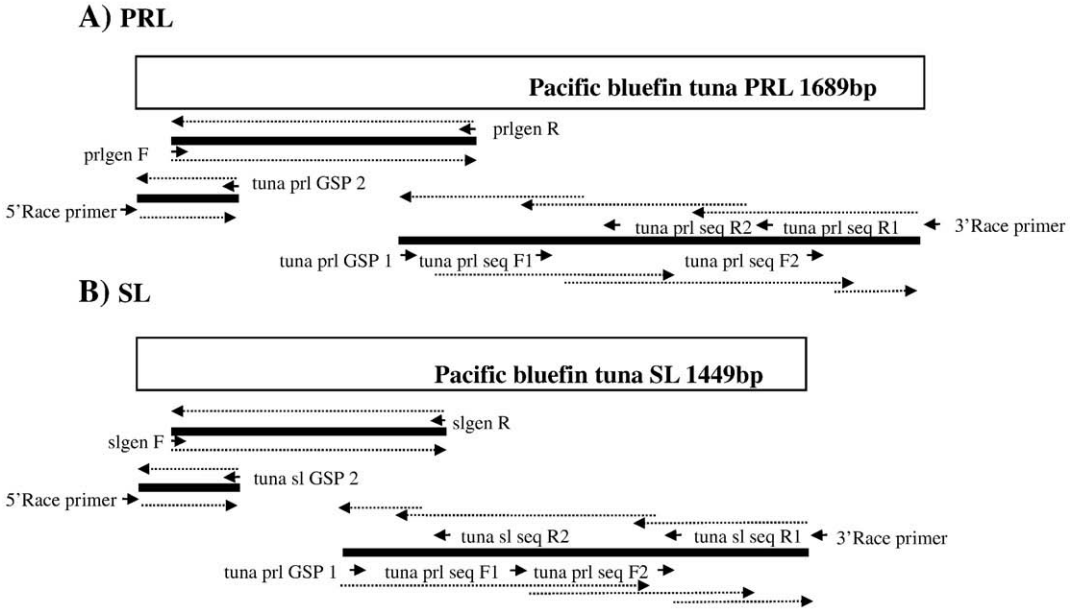

Fig. 1. DNA sequencing strategy for cDNAs of PRL and SL in PBT. Each short arrow indicates a designed primer as shown in Table 1. The 5′ and 3′ primers are from Gene Racer (Invitrogen). Bold lines show fragments obtained by PCR. Dotted arrows show sequenced areas and the direction of PCR fragments.