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Effects of fasting and refeeding on gene expression of *slc15a1a*, a gene encoding an oligopeptide transporter (PepT1), in the intestine of Mozambique tilapia

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ABSTRACT

The tissue distribution of *slc15a1a*, a gene that encodes an oligopeptide transporter, PepT1, and its response to fasting and refeeding were investigated in the intestinal epithelium of Mozambique tilapia for a better understanding of its role on nutrient absorption. The *slc15a1a* was predominantly expressed in the absorptive epithelia of the anterior part of the intestine, suggesting that digested oligopeptides are primarily absorbed in the anterior intestine. The response of *slc15a1a* to fasting was evaluated at 1, 2, 4, 7 and 14 days after the last feeding. Fasting revealed a biphasic effect, where short-term fasting significantly upregulated *slc15a1a* expression and long-term fasting resulted in downregulation. The expression level continued to decrease and fell below the pre-fasted level from day 4 to 14. Proximal (the hepatic loop, HL) and distal parts (the proximal major coil, PMC) of the anterior intestine showed different magnitudes of responses to fasting; *slc15a1a* expression in the PMC showed greater upregulation and downregulation than that in the HL. Refeeding significantly stimulated *slc15a1a* expression at day 3, although the expression did not exceed the pre-fasted level. Observed responses of *slc15a1a* to fasting and refeeding suggest that the expression level of this gene can serve as a sensitive indicator of the changes that may occur in altering nutritional conditions. These findings contribute to a better understanding of the role of PepT1 in nutrition and of the complex mechanisms underlying the absorption of oligopeptides and amino acids in the intestine, and may lead to development of possible means to manipulate the absorption processes for the improvement of growth and other metabolic and physiological conditions in fish.

1. Introduction

Utilization of exogenous compounds, which are selectively absorbed from extraorganismal environments, is fundamental function for every living body. In animals, nutrient absorption is completed by the gastrointestinal tract. Animals metabolize various kinds of nutrients, and in particular, amino acids are essential for synthesis of proteins and other non-protein nitrogenous molecules required for somatic growth and other physiological processes. Especially in teleost species, amino acids are more important nutrients compared with those in other vertebrates due to their higher dietary requirement of protein (Clements and Raubenheimer, 2006). Essential amino acids which cannot be synthesized by metabolic pathways should be acquired from the dietary proteins that are hydrolyzed into smaller chain peptides and amino acids in the gastrointestinal tract. The resulting peptides and amino acids are selectively absorbed from the lumen to the enterocytes, where most peptides are further hydrolyzed into amino acids before they are transported to the circulatory system (Ganapathy et al., 2000). Oligopeptide transporter 1 (PepT1), also known as solute carrier family 15 member 1 (SLC15A1), is found in the brush border membrane of the intestinal epithelia (Reviewed by Daniel, 2004). The PepT1 is responsible for absorption of di- and tri-peptides from the lumen to the enterocytes (Leibach and Ganapathy, 1996), while amino acid absorption is mediated by different types of amino acid transporters (Mailliard et al., 1995). Unlike amino acid transporters that are highly specific to electrochemical characteristics of amino acids, the low-affinity/high-capacity PepT1, transports nearly all oligopeptides such as neutral, acidic or basic peptides (Daniel, 2004; Daniel and Kottra, 2004; Vig et al., 2006). Moreover, PepT1 is also shown to interact with some medically important drugs such as β-lactam antibiotics, amino peptidase and angiotensin-converting enzyme inhibitors (Rubio-Aliaga and Daniel, 2002, 2008).

PepT1 characteristics, functional properties and transport mechanisms have been widely demonstrated in mammals including humans (Adibi, 1971, 1997; Ferraris and Diamond, 1989; Mailliard et al., 1995;

Abbreviations: DMC, distal major coil; GL, gastric loop; HL, hepatic loop; PepT1, peptide transporter 1; PMC, proximal major coil; TS, terminal segment; *slc15a1a*, solute carrier family 15 member 1.

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Reeds et al., 2000; Vig et al., 2006; Wu, 2009), rats (Karasov et al., 1987; Ogihara et al., 1999; Shiraga et al., 1999; Fei et al., 2000; Ihara et al., 2000; Rome et al., 2002; Howard et al., 2004), chicken (Chen et al., 2002; Gilbert et al., 2008; Miska et al., 2015), pigs (Zhang et al., 2013), and sheep (Matthews, 1983; Matthews et al., 1996; Pan et al., 2001). In fish, earlier evidences showed peptide transport in the brush border membranes of the fish intestine (Bogé et al., 1981; Reshkin and Ahearn, 1991; Verri et al., 1992; Thamotharan et al., 1996a, 1996b; Maffia et al., 1997). PepT1 has since been cloned and functionally characterized in several species including zebrafish *Danio rerio* (Verri et al., 2000, 2003), Asian weatherloach *Misgurnus anguillicaudatus* (Gonçalves et al., 2007), Atlantic cod *Gadus morhua* (Rønnestad et al., 2007), sea bass *Dicentrarchus labrax* (Terova et al., 2009), Atlantic salmon *Salmo salar* (Rønnestad et al., 2010), killifish *Fundulus heteroclitus macrolepidotus* (Bucking and Schulte, 2012), grass carp *Ctenopharyngodon idella* (Liu et al., 2013), yellow perch *Perca flavescens* (Kwasek et al., 2012) and icefish *Chionodraco hamatus* (Rizzello et al., 2013). Studies on functional characterizations showed that fish PepT1 is molecularly diverse compared to the mammalian counterparts. This might be related to physiological adaptation of fishes to their environments (Verri et al., 2003; Romano et al., 2014). Indeed, previous studies showed that PepT1 expressions changes at different life stages of some fish (Verri et al., 2003; Amberg et al., 2008; Ahn et al., 2013). Also, PepT1 tissue distribution differs among fish species (Verri et al., 2003; Terova et al., 2009; Rønnestad et al., 2010; Ahn et al., 2013). Moreover, variation in PepT1 expressions was observed in fish exposed to different environmental conditions (Bucking and Schulte, 2012; Rimoldi et al., 2015).

Variation in PepT1 expressions in response to nutritional condition was also demonstrated among fish species. In fact, malnutrition in the form of fasting induced differences in the response period and PepT1 expression patterns in killifish (Bucking and Schulte, 2012), sea bass (Hakim et al., 2009), zebrafish (Koven and Schulte, 2012). Indeed, malnutrition could result in disruption of metabolic and physiological homeostasis, which is detrimental to nutrient transport. On the other hand, resumption of feeding after fasting resulted to upregulation of PepT1 expression to a level higher than the pre-fasted, thereby supporting its role in promoting compensatory growth in some fish (Terova et al., 2009; Verri et al., 2011; Bucking and Schulte, 2012; Koven and Schulte, 2012). Furthermore, dietary modifications revealed changes in fish PepT1 expressions, indicating that diet could regulate the peptide and amino acid absorption and homeostasis in fish (Amberg et al., 2008; Bakke et al., 2010; Ostaszewska et al., 2010a, 2010b; Kwasek et al., 2012; Liu et al., 2014; Rimoldi et al., 2015; Cai et al., 2015; Borey et al., 2016).

In spite of the increasing studies on PepT1 in fish, the effect of nutrient condition and dietary regulation still needs substantial investigation to improve understanding of its role in nutrition. Previous studies have shown that amino acids are absorbed more efficiently in the form of small peptides than of free amino acids in the gastrointestinal tract (Bogé et al., 1981; Reshkin and Ahearn, 1991; Thamotharan et al., 1996a, 1996b), suggesting a potential role of PepT1 in the optimization of amino acid absorption and thus, its immense importance in fish nutrition (Bucking and Schulte, 2012). The aim of this study is to evaluate the regional distribution, and to investigate the effect of nutritional conditions (fasting and refeeding) on the expression of *slc15a1a,* a gene that encodes PepT1 in the intestinal epithelium of Mozambique tilapia *Oreochromis mossambicus*. Peptide transport has been demonstrated in tilapia (Thamotharan et al., 1996a, 1996b) but to the best of our knowledge no studies have been conducted on PepT1 and its response to nutritional condition in this species. Mozambique tilapia is one of the most farmed tilapia species in aquaculture and is becoming one of major sources of protein for humans. Knowledge acquired from this study would contribute to the better understanding of the role of PepT1 fish nutrition and the development of possible means to improve amino acid absorption, and therefore to optimize growth and other metabolic and physiological processes in fish.

2. Materials and methods

2.1. Fish

Mozambique tilapia *Oreochromis mossambicus* (100–250 g) were stocked in a 3000-*l* tank with recirculating freshwater at 25 °C. Fish were fed once a day on commercial carp chow (Nihon Haigo Shiryo, Kanagawa, Japan). In the following fasting and refeeding experiments, six fish were reared with or without feeding in one plastic tank (100*l*) with recirculating freshwater at 25 °C, and one tank was allotted to one sampling time point. Fish were acclimated to tank condition for 1 week, and were fed once a day at 10:00 in the morning. Prior to dissection and tissue collection, fish were anaesthetized with 2-phenoxyethanol (0.1%). All experiments were performed following the principles and procedures approved by the Institutional Animal Care and Use Committee of the University of Tokyo.

2.2. Tissue sampling and cDNA synthesis

The anterior part of the intestine was used for cDNA cloning of *slc15a1a*. The brain, gill, heart, liver, kidney, esophagus, stomach, intestine (divided into five segments), rectum, muscle and skin were subjected to tissue distribution analysis for *slc15a1a*. The collected tissues were immersed in RNA extraction reagent (ISOGEN, Nippon Gene, Tokyo, Japan). Total RNA of each tissue was extracted according to manufacturers' instruction. The amount of total RNA in each sample was set to 2 μg. After DNase I (Roche Diagnostics, Basel, Switzerland) treatment, RNA was reverse-transcribed to single stranded cDNA using the high capacity RNA-to-cDNA Kit (Thermo Fisher Scientific, Waltham, MA) or SMARTer RACE 5′/3′ kit (Takara, Otsu, Japan) according to manufacturers' instructions. For the fasting and refeeding experiments, the intestine was divided into five segments as described in Smith et al. (2000): the hepatic loop (HL), proximal major coil (PMC), gastric loop (GL), distal major coil (DMC), and terminal segment (TS). For histological analysis, the HL and DMC were fixed in 4% paraformaldehyde (PFA) in 0.01 M phosphate-buffered saline (PBS) for 16 h, and stored in absolute methanol at −20 °C until use.

2.3. cDNA cloning

Degenerate PCR for *slc15a1a* was carried out using a following primer pair: *slc15a1a*-df (GCITTYGGNGGNGAYCARTT) and *slc15a1a*-dr (CCARAACATIGGNARNGGDAT). PCR was conducted in a final volume of 20 μL containing 10× PCR buffer (Takara), 200 μM of dNTPs (Takara), 0.5 U Taq DNA polymerase (Ex Taq, Takara), 100 pmol of each primer pair, and an appropriate amount of cDNA template of sample tissues of tilapia. The PCR products were ligated into pGEM T-easy (Promega, Madison, WI, USA) and sequenced by a DNA sequencer, ABI PRISM 310 (Life Technologies). Sequence data were analyzed with ATGC software (Genetyx, Tokyo, Japan). After determination of the partial cDNA sequences of *slc15a1a*, 3′- and 5′-rapid amplification of cDNA ends (RACE) were carried out to extend sequence information at 3′ and 5′ ends with adaptor primers in SMARTer RACE 5′/3′ kit and the following gene-specific primers: 5′-RACE(CAGAGTGGAGATGAGACTTCCAGC) and 3′-RACE(GCTCAGGTGAAGATGGCATTGAAG). Topology prediction for the deduced amino acid sequence of tilapia *slc15a1a* was performed with the software TMHMMfix (http://www.sbc.su.se/ ~melen/TMHMMfix/).