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Oral lipase activities and fat-taste receptors for fat-taste sensing in chickens

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ABSTRACT

It has been reported that a functional fat-taste receptor, GPR120, is present in chicken oral tissues, and that chickens can detect fat taste in a behavioral test. However, although triglycerides need to be digested to free fatty acids to be recognized by fat-taste receptors such as GPR120, it remains unknown whether lipase activities exist in chicken oral tissues. To examine this question, we first cloned another fat-taste receptor candidate gene, *CD36*, from the chicken palate. Then, using RT-PCR, we determined that *GPR120* and *CD36* were broadly expressed in chicken oral and gastrointestinal tissues. Also by RT-PCR, we confirmed that several lipase genes were expressed in both oral and gastrointestinal tissues. Finally, we analyzed the lipase activities of oral tissues by using a fluorogenic triglyceride analog as a lipase substrate. We found there are functional lipases in oral tissues as well as in the stomach and pancreas. These results suggested that chickens have a basic fat-taste reception system that incorporates a triglycerides/oral-lipases/free fatty acids/GPR120 axis and CD36 axis.

1. Introduction

Although fats and lipids constitute one of the three main nutrients in food, along with carbohydrates and proteins, the molecular mechanism underlying the lingual sensation of fats has not been elucidated well in chickens. Detailed elucidation of this mechanism would provide the basic information of taste sense in chickens, and would be useful for developing new feedstuffs. Previously, we cloned the fatty acid receptor gene, *G-protein coupled receptor 120* (*GPR120*), from chicken oral tissues, and we found that chicken GPR120 (cGPR120)-expressing cells were activated by oleic acid and linoleic acid, which are the main fatty acids in chicken feeds, and that chickens show a preference for corn oil, which contains large amounts of oleic acid and linoleic acid, in behavioral tests [1].

GPR120-knockout mice exhibit a decreased gustatory nerve response to oral free fatty acids (FFA) stimulation, and display a lower preference for linoleic acid than their wild-type counterparts [2]. Moreover, knockout mice of the cluster of differentiation 36 (CD36), which is a fatty acid transporter, exhibit defects in their preference for and ability to detect linoleic acid in a 5 min licking test [3]. Both GPR120 and CD36 are known to be expressed in taste cells in rodents and humans [4–7]. Based on these facts, it is highly possible that both GPR120 and CD36 are fat-taste receptors in rodents and humans. However, in chickens, the expression patterns of these fat-taste receptor candidates have not been analyzed.

Because the agonists of GPR120 and CD36 are long-chain fatty acids (LCFA), the triglycerides contained in foods or feeds must be digested to LCFA by oral lipase in order to bind to oral GPR120 and CD36. Kawai et al. elucidated that functional lingual lipase is present in rat oral tissue, based on the finding that a small percentage of the total triglycerides were digested to FFA only 1 s after being dropped onto rat circumvallate papillae [8]. It has also been reported that perceived fat taste was decreased by the inhibition of oral lipase activity in humans [9]. These evidences suggest that the presence of fat-taste sensors and oral lipase activities are important to detect fat taste. In chickens, however, there is currently no evidence as to whether lipase activities exist in the oral tissues.

In the present study, to elucidate a part of the fat-taste mechanisms in chickens, we focused on the expression patterns of two fat-taste sensor candidates, GPR120 and CD36, and several lipase genes in the oral and gastrointestinal tissues. We further focused on the oral lipase activities in chickens. We showed that the two fat-taste sensor genes and lipase genes are expressed in the oral

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tissues, and that chickens also have functional lipases in oral tissues. These findings suggest that chickens have the fundamental mechanisms to detect fat taste in oral tissues.

2. Materials and methods

2.1. Animals

Rhode Island Red strain 1-2-wk-old chicks were used for this research. The study was carried out according to the Guide for Animal Experiments issued by Kyushu University, the Law Concerning the Human Care and Control of Animals (Law No. 105; October 1, 1973), and the Japanese Government Notification on the Feeding and Safekeeping of Animals (Notification No. 6; March 27, 1980).

2.2. Chicken CD36 construction

Total RNA was isolated from the palate of chicks, and first-strand cDNA was synthesized by reverse transcription. Then, deduced open reading frames (ORFs) of chicken *CD36* (*cCD36*) were amplified and sequenced. The polymerase chain reaction (PCR) primers were designed based on the genome assemblies of the *cCD36* (NM_001030731.1). The primers were designed as exon-spanning. The PCR products of the ORFs were subcloned into the pcDNA3.1(+) expression vector by using an In-Fusion HD Cloning Kit (Takara Bio, Otsu, Japan). The entire sequence of *cCD36* was confirmed.

2.3. RT-PCR analysis

Total RNA was isolated from the brain, oral tissues (upper rostrum, lower rostrum, palate, oral-cavity floor, and tongue tip), and gastrointestinal tissues (ingluvies, stomach, gizzard, duodenum, jejunum, ileum, and colon), using ISOGEN II (Nippon Gene, Tokyo) according to the manufacturer's instructions. The first-strand cDNA was synthesized by reverse transcription, with the application of 1.0 μg total RNA with or without reverse transcriptase using a PrimeScript RT reagent kit with gDNA Eraser (TaKaRa Bio, Otsu, Japan) following the manufacturer's protocol. Primers were designed with the aid of the nucleotide database of The US National Center for Biotechnology Information and are shown in Table 1. The PCR mixture had a total volume of 10 μl and consisted of ultrapure water, 5 × PrimeSTAR GXL Buffer, dNTP Mixture (2.5 mM each), primer forward (0.2 μM), primer reverse (0.2 μM), cDNA (1–6 ng), and PrimeSTAR GXLDNA Polymerase (0.25 units) (TaKaRa Bio). PCR reactions were conducted under the following conditions: 40 cycles of 98 °C for 10 s, 55 or 60 °C for 15 s, and 68 °C for 1 min/1 kb. Five-microliter PCR products were electrophoresed on a 1.8 or 2.3% TAE agarose gel.

2.4. Lipase assay

Chicks were euthanized, and the palate, tongue tip, rostrums (mixture of the upper and lower rostrum), stomach, and pancreas were immediately collected. Two-hundred milligram portions of each of these tissues were weighed out and minced into small pieces. The tissues were then rinsed with cold PBS and homogenized in buffer (1 mL of cold, 20 mM Tris, pH 7.5, and 150 mM NaCl). After centrifuging at 10,000 × g for 10 min at 4 °C, the supernatants were collected and used for lipase assays. We measured the lipase activities of these tissues by using a Fluorometric Lipoprotein Lipase (LPL) Activity Assay Kit (Cell Biolabs, San Diego, CA) according to the manufacturer's instructions. In addition to LPL, this kit can also be used to detect endothelial and hepatic lipase activity. The kit utilizes a fluorogenic triglyceride analog as a lipase substrate. When uncleaved, the substrate remains in a non-fluorescent, quenched state; however, upon hydrolysis by lipase, a fluorescent product is produced. We measured the fluorescent product using an Infinite® 200 PRO fluorescence microplate reader (Tecan Group, Männedorf, Switzerland).

2.5. Statistical analysis

Values are expressed as the means ± SE. The lipase activity data was analyzed by Tukey test. Statistics were calculated with an IGOR Pro software package (Version 6.34J; WaveMetrics, Portland, OR); differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Cloning of CD36 from chicken palate

First, we detected *cCD36* mRNA in the chicken palate by RT-PCR (data not shown). Then, we cloned the *cCD36* gene from the chicken palate. After amplification in *E. coli*, we confirmed that the cDNA sequences of the cloned *cCD36* matched that of the *Gallus gallus* genomic sequences of the NCBI database (NM_001030731.1) except for two bases (Fig. 1). The translated product of the cloned *cCD36* gene matched the database (NM_001030731.1) except for two amino acids.

3.2. RT-PCR of CD36 and GPR120 in oral and gastrointestinal tissues

We analyzed the expressions of *CD36* and *GPR120* in three chicks by RT-PCR. Representative data of one chick are shown in Fig. 2 mRNAs of *GPR120* were expressed in the palate, oral-cavity floor, ingluvies, stomach, gizzard, duodenum, jejunum, ileum, and colon. mRNAs of *CD36* were expressed in the upper rostrum, lower rostrum, palate, oral-cavity floor, tongue tip, ingluvies, stomach, gizzard, duodenum, jejunum, ileum, and colon. We observed no bands for either of the two genes in the negative control reactions without reverse transcriptase (RT-) in any of the tissues examined (data not shown).

Table 1
Primers used for the RT-PCR.

Target gene	Abbreviation	Accession no.	Primer forward	Primer reverse	Product size (bp)
G-protein coupled receptor 120	*GPR120*	XM_003641481.2	AGTGTCACTGGTGAGGAGATT	AACACAATGAGGGCTCGGAA	254
Cluster of differenciation 36	*CD36*	NM_001030731.1	TGCACCCTGTCAAAGGAGAG	GTTCAAAACGGGCAGCGTTA	389
Lipoprotein lipase	*LPL*	NM_205282.1	CCGATCCCGAAGCTGAGATGA	AGAGCCTCGAGTGTAGGTGT	572
Endothelial lipase	*LIPG*	XM_424455.5	TTGCCCACCAACTCTACACC	AGGGTCCAAGCCTGTGATTC	206
Lipase I	*LIPI*	XM_416675.4	AGGAGGAGTCATGAATTGCAGAA	CCGCTGGATCAAGACCTGTA	726
Lipase H	*LIPH*	XM_015277163.1	TCAATTCGACAGCCTCCAAGT	AGTGGTAGAGGCAAAATGGCT	822
Carboxyl ester lipase	*CEL*	NM_001012997	ATCACTGCGAGCGATGTCTA	GGTTGTCACCCCACAACTCT	109
Adipose triglyceride lipase	*ATGL*	NM_001113291.1	TGGACTCCGCTTGGAACATC	TGCCTCCAAAAGAGCTTGGT	935
β-actin		NM_205518.1	CCGGACTGTTACCAACACC	CACTTTACTCCTAGACTGTGG	515

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