Basic studies on taurine synthesizing ability and its synthetic pathways in Japanese flounder Paralichthys olivaceus and common carp Cyprinus carpio

[博士過程]

Doctoral Course

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博士学位論文の要約

Taurine is important for early growth, development, visual system, neurotransmission, reproduction, and osmoregulation in fish. However, understanding of regulatory mechanism and synthetic pathway of taurine in fish is limited. In previous studies, commonca carp *Cyprinus carpio* and Japanese flounder *Paralicthys olivaceus* were suggested to possess different potential to synthesize taurine from its precursor in their body. This difference could be due to limited expression level of taurine synthesizing enzymes. However, another explanation for different ability of taurine synthesis in the two species is use of different taurine synthesizing pathways. So far, there are three different taurine syntheic pathways are proposed in animals; cysteine sulfinate pathway where cysteine dioxygenase (CDO) and cystiensulfinate decarboxylase (CSD) works for production of cysteine sulfinate and hypotaurine, respectively. Cysteamine pathway where cysteamine/aminoethathiol dioxygenase (ADO) works for production of hypotaurine from cysteamine. Lastly, cysteic acid pathway where cystic acid was directly converted into taurine by cysteic acid decarboxylase (CAO) or CSD. To examine involvement of these enzymes in diffirent taurine synthesizing patways, expression analysis of genes encoding these enzymes in test fish species can be done. However, CDO, CSD, ADO, and CAO have not been cloned in carp and flounder when the author started her study. Therefore, the first study cloned the gene sequence of these eyzmes responsible for taurine synthesis in Japanese flounder and common carp. In the previous studies, limited ability of taurine synthesis in Japanese flounder probably due to limitation of CSD activity is well documented in Japanese flounder. However, inconsistent results were reported in terms of taurine synthetic ability in common carp. Thereore, secondly, the author analyzed plasma taurine and related compounds contents in fish injected taurine precursors such as L-cysteine and cysteamine that are taurine precursor in cysteine sulfnate pathway or cystamine pathway, respectively. Finally, the author investigated growth and taurine and related compounds content in carp fed taurine, L-cysteine, L- methionine, and cysteamine-HCL supplemented diets.

Full length nucleotide sequences of CDO for Japanese flounder were all 1002 nucleotide bases with deduced amino acid sequence of 201 amino acids while CSD from Japanese flounder were all 2605 nucleotide bases with deduced amino acid sequence of 497

amino acids. While the cloned cysteamine dioxygenase (ADO) cDNA in common carp consists of 790 nucleotide bases with 260 deduced amino acid sequence. Three N-glycosilation sites were observed in common carp ADO suggeting supplementation of sugar chains is required for full activity of carp ADO. CDO and CSD gene trasncripts were observed in all the tested organs such as brain, eye, gills, gut, heart, kidney, liver, muscles, pancreas, pyloric caeca, spleen and stomach. Significantly higher CDO and CSD expression were observed in the liver and brain, liver, muscles and pyloric caeca, respectively in Japanese flounder. CDO1 expression level was generally higher than that of CDO2. CDO1/2 expression were observed all organs except intestine and highly expressed in muscle, hepatoopancreas, kidney, and gall bradder. Relativly high expression of CDO1 was observed in eye but only basal level of CDO2 expression was observed in carp. High CSD expression was observed in hepatopancreas, intestine and brain of carp. High expression of carp ADO were observed in brain, gill, intestine, hepatopancreas, spleen and kidney.

Injection of L-cysteine and cysteamine showed increase of plasma taurine after 2-8 and 8-12 h post injection, respectively. Increased taurine content in hepatopancreas was observed in fish injected cysteamine but not in L-cysteine. These results suggested that taurine was produced from L-cysteine and cysteamine but it was not accumulated when metablized from L-cysteine.

For the feeding trial, juvenile common carp were fed one of nine diets: a basal diet supplemented without sulfur compound (control); a basal diet supplemented with 1.0% or 1.5% cysteamine hydrochloride (CSH); 1.5% or 3.0% cysteine; 1.0% or 1.5% methionine; and 0.5% or 1.0% taurine for 30 d. The 1.0% and 1.5% CSH supplementation caused growth retardation and deformities in the fish. All treatments increased carcass taurine levels (18.5-86.9 g/kg). The highest whole body taurine content was observed in fish fed the 1.5% CSH-supplemented diet. CDO was downregulated by cysteine and 0.5% taurine but upregulated by 1.5% CSH. CSD was downregulated by cysteine, methionine, and CSH. ADO was downregulated by methionine, cysteine, and 0.5% taurine but upregulated by CSH. Somatostatin 14 was upregulated by CSH. Insulin-like growth factor-1 was upregulated by 1% taurine and cysteine but downregulated by 1.5% CSH. The present study suggests that the cysteamine pathway is mainly responsible for taurine synthesis in common carp.