論 文 の 内 容 の 要 旨

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論文題目 Studies on molecular mechanisms of lipid metabolism in Japanese flounder *Paralichthys olivaceus* and red seabream *Pagrus major*
（ヒラメ *Paralichthys olivaceus* およびマダイ *Pagrus major* における脂質代謝メカニズムに関する研究）

Lipid stored tissue plays a major role in free fatty acid supply for whole body energy homeostasis. The lipid in the form of triacylglycerol (TAG) is hydrolyzed by catalytic enzymes into free fatty acid (FFA) and glycerol. Hormone-sensitive lipase (HSL) is one of those catalytic enzymes which have an important role in lipolysis in mammalian adipose tissue. In fish, lipid accumulation is mainly deposited in three organs, adipose tissue, liver and skeletal muscle. Different fish species are likely to deposit lipid in the different types of organs, known as “tissue- and species-specific” manners. However, the process of HSL-mediated lipolysis in fish is poorly documented and remains unclear, which leads to misunderstanding and contradictory in our knowledge of lipid metabolism.

In order to clarify the function of fish HSLs, HSL cDNAs were cloned from two different species: Japanese flounder *Paralichthys olivaceus*, mainly stored their lipid in inclinator muscle of fin, and red seabream *Pagrus major*, mainly in visceral adipose tissue. The full-length cDNAs of two HSL genes were determined and designated as HSL1 and HSL2. In Japanese flounder, HSL1 and HSL2 consisted of 2,922 bp and 2,832 bp, respectively, while 2,955 for HSL1 and 2,723 bp for HSL2 were observed in red seabream. Additionally, proteins encoding HSLs were 702 and 837 amino acids in Japanese flounder, and 716 and 874 amino acids in red seabream, respectively. The molecular mass of Japanese flounder showed that the HSLs protein

migrated on SDS-PAGE exhibited an apparent molecular mass of approximately 96 kDa of HSL1 and 125 kDa of HSL2. On the other hand, HSL1 protein of red seabream has molecular mass of 98 kDa. However, HSL2 protein was not observed in red seabream. The deduced amino acid sequences of HSL1 and HSL2 genes shared 58.7% identity in Japanese flounder and 55% identity in red seabream. The identity of HSL1 and HSL2 in both of Japanese founder and red seabream showed about 57- 89%, and 60-73% with HSLs from rainbow trout *Oncorhynchus mykiss*. The multiple amino acid alignment with other species clearly showed that HSLs of the present study were comprised of two major domains; N-terminal and C-terminal, which were separated by glutamine and aspartate (Gln316 and Asp317 in Japanese flounder and, Gln322 and Asp323 for HSL1, and Gln355 and Asp356 for HSL2 of red seabream, respectively). Three amino acid residues comprising the catalytic triad were conserved in all HSLs. The multiple alignments showed that both Japanese flounder and red seabream HSLs also have several serine residues aligned with the potential phosphorylation sites of rainbow trout HSLs, but these were misaligned with the rat and human HSL of phosphorylation sites. Several phosphorylation motifs of mammalian PKA (R/K-R/K-X-pS/T or R/K-R/K-X-X-pS/T) were also conserved between both species.

Tissue distribution of HSLs performed by RT-PCR found that the HSL transcripts of the Japanese flounder genes were abundant in the inclinator muscle of fin, liver, and skeletal muscle, whereas the highest transcripts were observed in adipose tissue and gonad in red seabream. The relative mRNA levels revealed that the transcripts of HSL1 and HSL2 genes were broadly expressed in all tested tissues. The relative mRNA levels of HSL2 were lower than HSL1 in both Japanese flounder and red seabream, suggesting that HSL2 has a minor function in the hydrolysis of stored lipid. In addition, the relative mRNA levels and HSL proteins were accumulated in the different tissues, being significantly higher in inclinator muscle of fin for Japanese flounder, whereas the highest HSLs mRNA expression was observed in adipose tissue and gonad for red

seabream. HSL protein further showed that HSL1 immunoreactive bands were observed in adipose tissue and gonad. The present data confirm that the expression of HSL is tissue- and species-specific.

The present study found that the inclinator muscle of fin contained high amount of lipid in adipocytes aligned along the muscle fiber cells as revealed by oil red O staining. *In situ* hybridization showed that HSL1 transcripts were accumulated in the peripheral region of adipocyte in the inclinator muscle of fin in Japanese flounder. HSL2 of Japanese flounder and HSLs of red seabream were not observed in this study. Total lipid from inclinator muscle of fin and skeletal muscle were subjected to thin layer chromatography (TLC) in order to characterized lipid class compositions in the tissues. Cholesteryl esters and FFA were detected in the inclinator muscle of fin, but were not found in skeletal muscle. Accordingly, the present study suggests that adipocytes in the inclinator muscle of fin are lipid storage sites, which would possibly release FFAs for the fin continuous movement through the HSL-mediated lipolysis in Japanese flounder.

The effects of nutritional and endocrine regulations on HSL mRNA expression were examined using red seabream as a model organism. For nutritional regulation experiment, red seabream were fasted for 7 days and then refed for three days. HSL mRNAs in both adipose and liver tissues of fasted red seabream were rapidly increased from day 4 until day 7. Furthermore, HSL1 and HSL2 mRNA expressions in adipose tissue was higher than the expression found in liver tissue. Fasting resulted in depletion of stored lipid in mesenteric fat and liver, and this action was reversed to normal state by re-feeding. Fasting condition increased the expression of HSL1 mRNA to a greater extent than that HSL2 mRNA. The present findings indicate that fasting condition associated with lipid depletion is accompanied by increasing HSL expression.

Sliced tissues of three organs including adipose tissue, liver and skeletal muscle of red seabream were incubated with 3 concentrations of insulin and 4 different time ranges. HSL1 and HSL2 mRNA expression levels were

suppressed by insulin treatment compared to control in both adipose and liver tissues in concentration and time dependent manners. These results also suggest that HSL would be inhibited during insulin incubation, owing to dephosphorylation of HSL. The suppression of HSL phosphorylation was due to a decrease of cAMP, caused by phosphodiesterase 3B activation via PI-3K activation by insulin. In contrast, the expression of HSL1 was inhibited, whereas HSL2 was dramatically increased in skeletal muscle. To study the induction of GH on HSL mRNA expression, HSL mRNA levels in liver and mesenteric fat were performed under incubation in the presence of 4 concentrations of GH for 1, 4 and 10 h. The results suggest that GH stimulates HSL expressions in liver and adipose tissue and linearly increases until 10 h incubation. The present studies clearly demonstrate that GH stimulates HSL mRNAs in both of time- and concentration-related manners.

In conclusion, Japanese flounder and red seabream possess HSL encoding mRNAs that express tissues in different tissue. Their transcripts and HSLs protein were ubiquitously expressed in various tissues. The HSL mRNA levels were markedly high in the inclinator muscle of fin for Japanese flounder, whereas highly expressed in adipose tissue and gonad where TAG, CE, and FFA were accumulated. The transcripts of HSL1 were localized in adipocytes of the inclinator muscle of fin. These results suggest that the adipocyte around the inclinator muscle of fin is a supplier of FFA in Japanese flounder, and that HSL-mediated lipolysis provides FFAs for the continuous and aerobic movement of fins. Fasting condition stimulated the expression of the two HSL mRNAs in major lipid depots in a tissue-specific manner. The HSL mRNAs, HSL1 and HSL2 are differentially expressed within and among tissues, and state of nutrition would modulate the pattern of HSL expression in part via hormonal regulations such as insulin and GH.