Lipoprotein Lipase Gene in Red Sea Bream; A Summarized Paper

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SUMMARY: Lipoprotein lipase (LPL) is a key enzyme of lipid metabolism. The elucidation of the structure, function and regulation of LPL is important for understanding the lipid metabolism in fish. Recently, we have cloned the LPL gene of red sea bream (*Pagrus major*) and characterized it by cDNA and genomic structure analysis. Subsequently, using the cloned sequence, gene expression has been investigated. We review in this report the features of red sea bream LPL gene. Red sea bream LPL gene spans 6.3kb of the genome and is organized into ten exons and nine introns. The deduced amino acid sequences showed high degree of similarities to the LPLs of other animals. In a 1.1kb 5' flanking region, the homologous sequences for the response elements of Insulin, glucocorticoid and thyroid hormone were detected. These results suggest the hormonal regulation of the LPL gene expression. Red sea bream LPL gene was expressed in various tissues including adipose tissue, heart, liver (hepatopancreas) and muscle. A fourteen-week feeding experiment was conducted to investigate the effects of feeding condition and dietary lipid level. The results suggest that LPL gene expression in visceral adipose tissue and liver is regulated in tissue-specific manner. The expression level in adipose tissue was down-regulated during starvation whereas it was up-regulated in liver. The effects of dietary lipid level on the gene expression were not observed. These results will facilitate further study on the function and regulation of the LPL in fish.

KEY WORDS: Lipoprotein lipase, LPL, gene, red sea bream, *Pagrus major*.

INTRODUCTION

Lipoprotein lipase (LPL) plays an important role in plasma lipoprotein metabolism. LPL hydrolyzes triacylglycerols present in plasma lipoproteins and supplies free fatty acids for storage in adipose tissue, or for oxidation in other tissues.¹⁾ Studies of mammalian and avian LPL have revealed that the LPL protein contains multiple functional domains required for secretion, glycosylation, catalysis, and lipid and heparin binding. These functional domains are highly conserved throughout evolution.²⁾

LPL is synthesized in various tissues including adipose tissue, heart and muscle. The synthesis of LPL is regulated in response to nutritional, hormonal and developmental states of animals. Quantitative RNA analyses revealed that a part of the regulation is due to the change of the gene expression level, and that the gene expression level is mediated according to the physiological state of animals.^{3,4,5,6,7,8,9)}

To understand the molecular mechanisms of lipid metabolism in fish, elucidation of structure, function and regulation of LPL are important. Biochemical approaches have been conducted on fish LPL,^{10,11,12)} and the cDNA sequences of zebrafish and rainbow trout LPL are available.^{13,14)} Recently, the red sea bream LPL gene was cloned in our laboratory and characterized by cDNA and genomic structure analyses. Using the cloned sequence, the gene expression was investigated. In this report, we review

the features of red sea bream LPL gene with recent progress. Parts of this study were submitted as ref (15) and (16).

STRUCTURAL FEATURE OF RED SEA BREAM LPL GENE

The details of this section were submitted as ref (15). The 2,946 bp of cDNA and 7,294bp of the genomic sequences of red sea bream LPL are available under the DDBJ accession number AB054062 and AB054063, respectively.

The red sea bream LPL gene spans a region of approximately 6.3kb of the genome and the coding sequence is organized into ten exons and nine introns. The red sea bream LPL gene encodes 511 amino acids and the deduced amino acid sequence showed high degrees of similarity to the LPL of other animals. The amino acid sequence of red sea bream LPL exhibited 49-51% identities to the sequences of mammalian,^{17,18,19)} avian⁴⁾ and rainbow trout LPL.¹⁴⁾

As indicated in studies in higher vertebrates, LPL contains multiple functional domains and these functional domains in LPL are postulated to be confined to specific exons^{20,21)}: for example, the signal peptide for secretion is assigned to exon1, the functional N-glycosylation site to exon2, catalytic serine and a lipid binding region to exon4, a putative heparin binding region to exon6. In comparison with mammalian LPL,^{22,23,24)} the red sea bream LPL

showed high degrees of similarity (Fig.1). There seems to be little difference in LPL function between red sea bream and mammals.

Fig. 1 Comparison of the LPL structure

SP: signal peptide, LBR: lipid binding region, HBR: heparin binding region, *: catalytic triad, Gly: potential N-glycosylation site and S-S: disulfide bond are indicated.

Within a 1.1kb of the 5' flanking region, homologous sequences for potential cis-regulatory elements were detected (Fig.2). These elements were predicted by homologies to the consensus sequences reported in mammalian genes.^{25,26,27,28,29} It remains to be determined if they are actually functional. The existence of the response elements for insulin, glucocorticoid and thyroid hormone suggests the hormonal regulation of LPL gene transcription. LPL synthesis and activation are mediated not only at transcriptional level, but also at posttranscriptional level.³⁰ To understand the regulation of LPL synthesis and activation, further analyses are required.

Fig. 2 Potential cis-regulatory elements in the red sea bream LPL gene 5' flanking region

CCAAT, Oct-1, the response elements for glucocorticoid (GRE), insulin (IRE), thyroid hormone (TRE) and cAMP (CRE) are indicated.

TISSUE-SPECIFICITY OF RED SEA BREAM LPL GENE

As mentioned in the *INTRODUCTION*, LPL gene is expressed in various tissues. To clarify the tissue-specificity of red sea bream LPL gene expression, the expression in visceral adipose tissue, heart, liver (hepatopancreas) and dorsal white muscle was investigated.

The total RNA was prepared from 72~93g fish, and the LPL gene expression levels were evaluated by competitive

RT-PCR method. Since the expression levels may be affected by the nutritional state in fish, the samples were prepared from fish in both fed (5hr post-feeding) and starved (48hr post feeding) conditions. However, in this experiment, the differences in the gene expression level between 5hr and 48hr post-feeding condition in each samples were not statistically significant.

Red sea bream LPL gene was expressed in all the examined tissues (Fig.3). Relatively higher levels of expression were observed in liver (48hr post-feeding) and adipose tissue (5hr post-feeding).

Fig.3 Tissue-specificity of the red sea bream LPL gene expression

The gene expression levels (mean $\pm$ S.E.) are indicated as % β actin. (n = 4)

NUTRITIONAL REGULATION OF THE GENE EXPRESSION

The LPL gene is regulated in response to nutritional state of animals. To investigate the effects of feeding condition and dietary lipid level, a 14-week feeding experiment was conducted. The LPL gene expression levels were compared between fed (5hr post feeding) and starved (48hr post-feeding) conditions, and between the control diet fed fish (LL: dietary lipid level 15.3%) and high lipid diet fed fish (HL: 24.6%) (Fig.4). In this study, we estimated the gene expression level in visceral adipose tissue and liver because these tissues are major sites of lipid deposition in fish.^{31,32} For the most part, this section was submitted as ref (16).

In comparison between the different feeding conditions (LL; fed and starved), in the adipose tissue, the expression level under starved condition was 6.2 fold lower than that of fed condition. The LPL gene expression in the adipose tissue was down-regulated during starvation. In the liver, the LPL gene expression seems to be up-regulated during starvation. The gene expression level in the liver under starved condition was 18.3 fold higher than that of fed condition. These results suggest the gene expression in adipose tissue and liver were regulated differently in a

tissue-specific and reciprocal manner during nutritional transition.

Fig. 4 Nutritional regulation of the red sea bream LPL gene expression
LL: low lipid (dietary lipid level: 15.3%), HL: high lipid (24.6%), *: significant difference ($P < 0.05$)
The gene expression levels (mean $\pm$ S.E.) are indicated as % β actin. (n = 4~6)

Our research group is interested in lipid metabolism and body lipid deposition in fish with reference to fat deposition in cultured fish. As reported previously, the dietary lipid level affects the body lipid deposition.³³⁾ In this study, to investigate the effects of dietary lipid level on the lipid metabolism, the LPL gene expression level was compared between in the control diet fed fish (LL) and high lipid diet fed fish (HL).

In comparison with the control diet fed fish (LL), any significant differences in the gene expression were not indicated in high lipid-fed fish (HL) (Fig.4). However, the LPL gene expression levels in the adipose tissue and the liver seems to be affected in response to the dietary lipid level; Under the fed condition, the LPL gene expression in the adipose tissue of the HL-fish was more than 2 fold lower than that of the LL-fish. Furthermore, the expression level in liver of HL-fish was higher than that of the LL-fish under the both fed and starved condition. (5.3 fold in fed and 1.4 fold in starved condition, respectively). To confirm the effects of dietary lipid level on lipid metabolism in fish, further studies are required.

DISCUSSION

Lipoprotein lipase belongs to the lipase gene family which includes hepatic and pancreatic lipase. These three lipases had diverged from a single ancestral gene throughout evolution, and share high degree of similarities each other.^{21,23)} We identified the lipase gene described in

this report as LPL by the sequence homologies to the LPL of other animals and the phylogenetic analysis (data not shown). With respects to the deduced amino acid sequence, the genomic organization, and the response of the gene expression in adipose tissue during starvation, red sea bream LPL showed similar characters to the mammalian LPL.

In this study, we investigated the structure and the gene expression of red sea bream LPL gene. Our results will facilitate further study of the function and regulation of the LPL in fish. In lipid metabolism, not only LPL but other lipases are involved. For better understanding of lipid metabolism in fish, other lipases, including hepatic and pancreatic lipase should be investigated.

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