Journal of Applied Biological Sciences 4 (1): 33-40, 2010
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

Distribution of Lipase Activity in Selected Tissues of Rainbow Trout (*Oncorhynchus mykiss*)

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Received : February 15, 2010
Accepted : April 15, 2010

Abstract

Lipases (EC 3.1.1.3.) are water-soluble enzymes that catalyze the hydrolysis of ester bonds in water–insoluble, lipid substrates. Although the distribution and properties of various lipases from many tissues of human, laboratory and domestic animals have been studied, little is known about the pattern of distribution and physiological roles of these enzymes in the rainbow trout. The aim of the present study was to examine and compare the distribution of lipase in different tissues of the rainbow trout. A selection of tissue samples was assayed for lipase using the olive oil splitting method for the determination of formed free fatty acids. Significant activity of this enzyme was found throughout digestive system, which provokes that the pyloric caecal mass has the highest specific activity of lipase followed by the liver, kidney, stomach, spleen, distal intestine, proximal intestine, gill, heart, abdominal muscle and brain. High activity of lipase in the pyloric caecal mass of rainbow trout indicates its primary role in fat digestion. However, its wide tissue distribution suggests that this enzyme might perform other functions beside gastrointestinal fat digestion. The lipase activity in pyloric caecal mass seems to be dependent to bile salts under our assay conditions, resulting in a significant activity in the presence of natural bovine bile. The results of this study will be discussed in terms of the involvement of lipase in several biochemical and physiological functions in this species.

Key words: lipolytic activity; pyloric caecae; bile salt; fish

INTRODUCTION

Lipases (EC 3.1.1.3.) are versatile enzymes that catalyze the hydrolysis of ester linkages in neutral lipids such as triacylglycerols. Lipases are ubiquitous throughout living organisms, and genes encoding lipases are even present in certain viruses [1, 2]. These lipolytic enzymes hydrolyze the acyl chains either at primary or secondary positions [3]. However, a few lipases do not show any positional specificity [4]. In addition to triacylglycerols, lipases are also known to degrade Tween and water-soluble and insoluble esters. There are reports of a few animal and microbial lipases that hydrolyze phospholipids [5-7]. Enzymatic activity of many lipases has been shown to be modulated by calcium [8] and bile salts [9]. Pancreatic triacylglycerol lipase (PTL) provides a mechanism to release fatty acids (FA) at low temperatures (12°C-18°C) and secreted into the small intestine where it hydrolyzes dietary fat [10]. Few reports are available on the digestive secretion of lipase in fish. The differential activity and expression of fish PTL is seen in other locations in addition to the traditional organ of its synthesis, the pancreas [9]. Borlongan (1990) [11] reported optimal activity for pancreatic and intestinal lipase of milkfish, at two different pH values. In case of rainbow trout, few reports have been done such as Léger et al. (1979) [12] whom purified a lipase and colipase in cecae, intestine, and liver. With respect to lipase activity in other carnivorous fish, such as rainbow trout, those usually consume fat-rich food [13] where high lipase activity has been detected. However, German et al. (2004) [14] found that monkeyface prickleback, *Cebidichthys violaceus*, had significantly higher lipase activity than a closely related carnivore, high cockscomb *Anoplarchus purpurescens*. Horn et al. (2006) [15] also did not found differences in lipase activity among four closely related herbivorous and carnivorous atherinopsid fishes. The PTL activity in the rainbow trout was reported twice that of the sturgeon [16].

Specifically, pancreatic lipase (triacylglycerol-acyl hydrolase EC 3.1.1.3) hydrolyzes triacylglycerols on the lipid-water-boundary surfaces of micelles; however, adsorption of the pancreatic lipase to the triacylglycerol boundary surfaces is inhibited by bile acid salts. Whereas, colipase can be bound to boundary surfaces covered by bile acids and to pancreatic lipase and eliminating the

inhibitory effect of physiologic concentrations of bile acid salts on the activity of lipase [17]. In this sense, most lipases require bile salts in order to change the three-dimensional structure from the inactive to the active state [9]. These are called specific lipases or bile-dependent lipases. However, several others do not require bile salts and these are therefore called non-bile-salt dependent or non-specific lipases [18, 19]. Our objective was to identify and to realize a partial biochemical characterization of lipase in tissues of rainbow trout adults.

MATERIALS and METHODS

Four healthy adult female rainbow trout (1405 ± 32 g mean weight) were used in this study. The animals were held under natural photoperiod in a flow-through system supplied with clear water. Water quality was monitored daily registering pH (7.20-7.45), temperature (13°C-16°C), and dissolved oxygen (7.45-8.25). Fish were fed 1% of their body weight once a day with our laboratory-made dry fish pellets (38.0% crude protein, 3.5% fiber, 12.0% crude fat, 10.0% ash, 1.0% phosphate, and 11.0% water content) for 30 days. After 12h of starvation, fish were anesthetized with the suspension of clove powder (200 ppm) in a 40 liter polyethylene tank and blood was withdrawn from caudal vein using a medical syringe (23G) and immediately transferred to an Eppendorf tube containing 2.7% dry ethylene diamine tetra-acetic acid (EDTA) disodium salt to prevent clotting. Plasma was separated from blood by centrifuging at 4° C for 10 min at 5000 ×g. After blood sampling the animals were killed by cutting of head and their tissues (stomach, pyloric caecal mass, distal intestine, kidney, liver, heart, proximal intestine, swim bladder, spleen, abdominal muscle, brain, gonads, and gill) were separated, stripped of fat and extraneous materials (blood and gut contents), washed with distilled water and then blotted with paper wool. Tissues were weighed and freezing with liquid nitrogen until biochemical analysis. Samples of frozen tissues were minced on a prechilled parceled pestle into small pieces that were vigorously mixed and homogenized with an electric homogenizer (Heidolph Instruments, GmbH & Co, Germany) with a 4 mm generator at a setting of 6 for 30 s; the process was performed on ice. For the homogenization, a 0.025 M sodium phosphate buffer, pH 7.2 was used at a proportion of 1 g tissue in 9 ml of buffer. The suspensions were centrifuged at 4000 ×g for 15 min at 4° C. The supernatants stored in aliquots (0.5–3.0 ml) at - 80°C and frozen supernatants were used as the source of enzyme.

Lipase was assayed as described previously elsewhere [20, 21] with some addition and modification. Briefly, the quantity of fatty acid released in unit time is measured by the quantity of NaOH required to maintain a constant pH 7. The reaction mixture consisted of distilled water, tissue homogenate, phosphate buffer solution (pH 7) and olive oil emulsion (Olitalia, Italia). A 50% emulsion of olive oil in water using 5 % acacia (w/v) (Sigma, St Lewis, Mo, USA) as the emulsifying agent was found to be suitable. The mixture was placed on a shaker at 150 rpm for 24h and incubated at 16 °C, a temperature corresponding to the average temperature of the collection sites. Subsequently, 96% ethanol and 2 drops of 1% phenolphthalein indicator dissolved in methanol 99.8% were added and titrated against 0.05 N NaOH until the appearance of permanent pink. A control was taken using enzyme source that was inactivated by heating (95°C) prior to addition of buffer and olive oil emulsion. The milliequivalent of alkali consumed per mg of protein is taken as a measure of the specific activity of the enzyme. Protein content of the supernatant solutions were determined by the Bradford method [22], using crystalline bovine serum albumin (BSA) (Sigma, St Lewis, Mo, USA) as the standard and reported as mg protein equivalent to BSA. All analyses were performed in triplicates. The effect of different volumes (5-40 µl) of natural bovine bile on the activity of lipase from pyloric caecal-mass crude extracts and the effect of 0.05 N NaCl on lipase specific activity of digestive system tissues was tested to identify partial characteristics of lipase activity in digestive organs.

Statistical procedures

Statistical analysis was performed by one-way analysis of variance and Duncan's test using the General Linear Models Procedure of SAS (SAS Institute, Inc., Cary, NC) software (*ver*. 8e) for multiple comparisons of the means of lipase activities. To evaluate the effects of bovine natural bile and NaCl on lipase activity, Student's *t* test was employed. Statements of significance were based on $p<0.05$ unless otherwise noted.

RESULTS

Lipase activity in 12 different tissues of rainbow trout is shown in Table 1. However, we found that soluble protein extracts made from rainbow trout heart, gill, swim bladder, stomach, intestine, kidney, brain, abdominal muscle and liver exhibited measurable lipolytic activity using the olive oil splitting method of determination of formed free FA at 16°C. All of the tissues studied contain lipase activity except gonads. The specific activity of lipase was significantly ($p_{ANOVA}=0.0243$) different among tissues. The pyloric caecal mass that contains diffuse tissues of exocrine pancreas had the highest activity of this enzyme. Liver, kidney, stomach and spleen had higher lipase specific activity than other tissues except pyloric caecal mass ($p<0.05$). Other tissues with relatively high lipase specific activity were the distal intestine, proximal intestine, gill and heart. Very low lipase specific activity was detected in abdominal muscle, brain and swim bladder.

The high distribution of lipase activity throughout gastrointestinal tract from stomach to distal intestine

Table 1. Mean (SEM) lipase specific and total activities in different tissues

Tissues	Specific Activity(Units/mg protein)	Total Activity (Units/ g tissue)
Stomach	0.77 (0.30) ^{b}	0.17 (0.06) ^{cd}
Pyloric caecal mass	7.74 (3.47) ^{a}	1.00 (0.27) ^{a}
Distal gut (Jejunum & Ileum)	0.52 (0.06) ^{b}	0.40 (0.01) ^{bcd}
Kidney	1.25 (0.26) ^{b}	0.43 (0.06) ^{bcd}
Liver	1.27(0.32) ^{b}	0.59 (0.10) ^{abc}
Heart	0.32 (0.13) ^{b}	0.18 (0.06) ^{dc}
Proximal gut (Duodenum)	0.37(0.01) ^{b}	0.68(0.05) ^{ab}
Swim bladder	0.11 (0.02) ^{b}	0.23 (0.09) ^{bcd}
Spleen	0.77 (0.17) ^{b}	0.67 (0.03) ^{ab}
Abdominal muscle	0.28 (0.10) ^{b}	0.09 (0.02) ^{d}
Brain	0.24 (0.06) ^{b}	0.50 (0.01) ^{bcd}
Gill	0.33 (0.01) ^{b}	0.17 (0.07) ^{cd}

Note: Values with different superscripts are significantly different ($p< 0.05$)

indicates that all parts of the digestive system involve in fat digestion. The activity of the lipase in extracts of stomach and pyloric caecal mass was not affected significantly ($p>0.05$) in the presence of NaCl (0.1 M). The salt-resistant lipase (i.e., non-LPL) activity was detected in intestine and liver (see Figure 1). The lipolytic activities of liver decreased significantly ($p<0.05$) in the presence of low NaCl concentration (0.1 M). Activities of lipase in the pyloric caecal mass, the main source of lipase, were affected positively by addition of natural bovine bile ($p_{ANOVA}=0.0001$) in comparison to bile-free controls. Enhancement of enzyme activity at different concentrations of natural bovine bile is shown in Figure 2. Lipase activity was not detected in plasma.

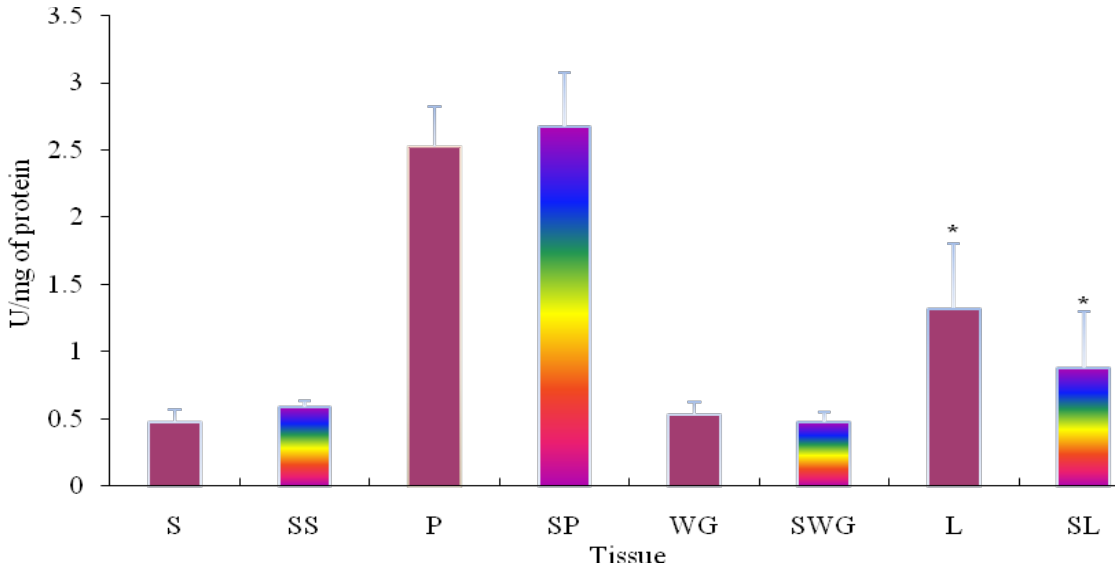

Figure 1. The effect of NaCl (0.1 M) on lipase specific activity of digestive system tissues

Note: S = stomach; SS = stomach in the presence of NaCl; P = pyloric caecal mass; SP = pyloric caecal mass in the presence of NaCl; WG = whole intestine; SWG = whole intestine in the presence of NaCl; L = liver; SL = liver in the presence of NaCl.*, $p<0.05$.

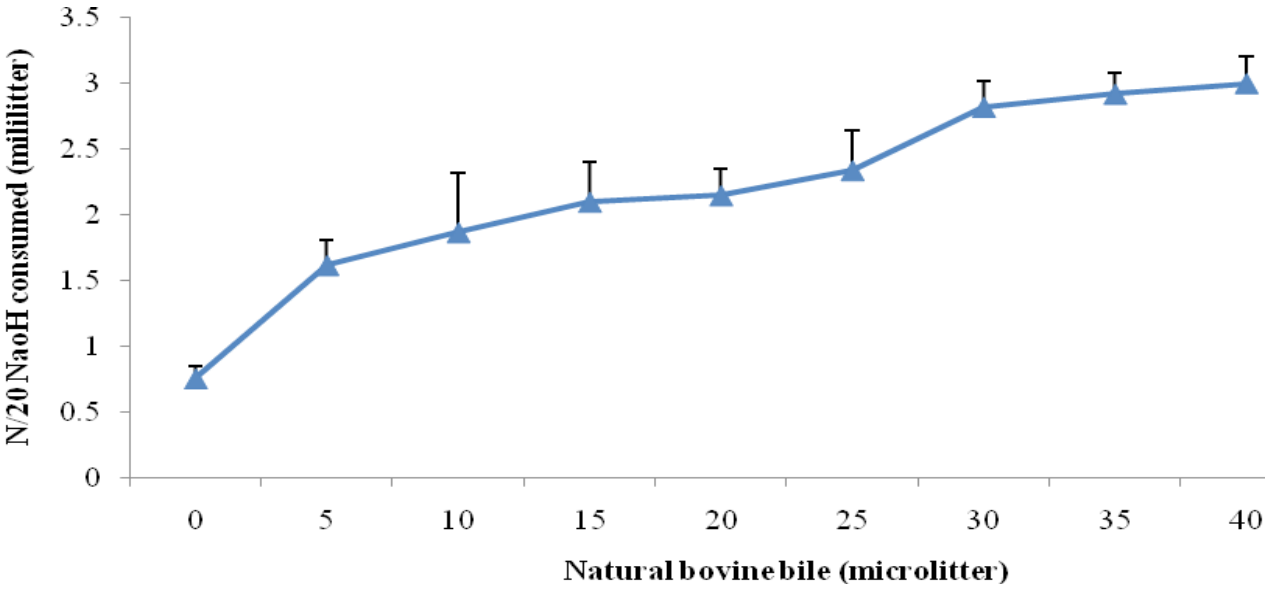

Figure 2. Effect of natural bovine bile on lipase activity of pyloric caecal mass in rainbow trout

DISCUSSION

Lipases form a rather diverse group of enzymes that can be divided into several classes, based on differences in their amino acid sequences, their tissue specificity of expression and their function [23]. In present study, lipase activity showed widespread distribution in rainbow trout tissues. Pyloric caecal mass had the highest level of this enzyme activity. Lipase from fish pancreas [18, 24, 25] and pyloric caecae of cod *Gadus morhua* [19] and tuna *Thunnus albacares* [26] have been purified and partially characterized. Gjellesvik et al. [19] purified a bile salt dependent lipase of defatted powder of cod pyloric caecae by combined affinity chromatography. Several studies have evaluated the activity of lipases during the larval stage of marine fish [27-30]. When the digestive system is fully developed, lipid digestion is supported by true lipases. In the present study, lipase activity was significantly higher in the pyloric caecal mass compared to the proximal intestine (duodenum) and distal intestine (jejunum and ileum). This is roughly in agreement with several reports that have shown that lipid digestion and absorption occurs in the proximal intestine [11, 31]. The striking feature of PTL in rainbow trout pyloric caecal mass was its increased activity in the presence of bovine natural bile and the present results suggest that bile-dependent lipases are the main enzymes involved in fat digestion. In contrast to our results, Para et al. [32] found a 60% reduction in enzyme activity in the presence of bile salts in bluefin tuna. Several studies have reported an inhibitory effect of bile salts on rat lipase activity [33, 34], indicating the presence of different lipases. In addition, Gjellesvik et al. [19] concluded that bile-dependent lipases are the only pancreatic enzymes involved in lipid digestion in cod. Extrapancreatic lipases of tongue, pharynx, esophagus and stomach evoke the hydrolysis of the fat contained in food [17]. Lipase activity was significantly higher in the caecal mass compared to the stomach. Preduodenal (gastric and lingual) lipases have been reported in the most domestic and laboratory animals [35].The gastric lipase activity in these species may be due to the feeding habits and constant lipid content in the diet. Moreover, the occurrence of lipase activity is more important in carnivorous fish as they feed on food rich in fat [13]. Although each of these lipase family members may play a role in fat metabolism, their expression may be varies both developmentally and by tissue. It has shown that the intestine of rats does indeed synthesize lipase almost identical to lipase of pancreatic origin, and that it is responsive to lipid feeding [36]. The preduodenal and pancreatic lipases are known to hydrolyze dietary fats in the lumen of the gastrointestinal tract of adult mammals and roughly in fish. Their combined action produces monoacylglycerols (MAGs) and FA that are readily absorbed by enterocytes lining the small intestine. The presence of sodium chloride has been found to be important to activate pancreatic lipase in rats [34] while we did not detect any significant effect of sodium chloride (0.1 M) on lipase activity in the extracts of digestive organs except liver which suggests that the lipolytic activity is not strictly due to clearing-factor (lipoprotein) lipase [21]. It seems that lipase activity of liver in rainbow trout sharing different properties with rat hepatic lipase (HL) in terms of salt resistance [37].

In the present study, lipase activity in brain was high on the basis of U/g wet weight, this finding indicates that the lipase activity plays some kind of roles in the rainbow trout brain. Diacylglycerol lipase was reported in the brain of rabbit, mice and cow [38, 39, 40]. In the present investigation, lipase activity has been demonstrated biochemically in the homogenate of spleens. Lipase activity in these homogenates was far less than one tenth

of the activity detected in the homogenates of pyloric caecal mass. It has been suggested that a lipolytic enzyme may play a role in cell-mediated immune reaction in mice [41].

Triacylglycerol may provide a reserve of fuel in most muscles, including those in which it does not serve as a major source of fuel for mechanical activity (e.g. vertebrate white muscle and the flight muscles of some insects). Rainbow trout abdominal muscle contains substantial lipase activity in comparison to many other tissues. Crabtree and Newsholm [42] reported the absence of triacylglycerol lipase activity from dogfish red muscle. Lipase activity in abdominal muscle may be due to LPL activity although, Magnoni and Weber [43] concluded that red muscle LPL is strongly activated by endurance swimming and rainbow trout have a high reserve capacity for hydrolyzing lipoproteins. LPL activity was reported in the skeletal muscle of rats [44]. LPL has been characterized in tissues such as red and white muscle, mesenteric fat, gonads and liver in fish [45-47]. The full nucleotide sequence for LPL from rainbow trout (*Oncorhynchus mykiss*) has been reported [48]. In contrast to mammals, LPL mRNA is expressed in liver of adult trout; this indicates that trout LPL carries out functions that HL has evolved to take over in mammals. In fish liver, lipase activity with an apparent heparin affinity similar to or even higher than LPL was found [45]. It is not clear whether the lipase activities in liver of fish are variants of HL. HL is present in liver and adrenals, but not in other tissues in male rats [49]. Fatty acids are an important fuel source for heart and skeletal muscle, providing over 70% of the energy needs for cardiac function [50, 51]. Thus it is likely that hearts are continuously generating a large amount of FA from triacylglycerol lipolysis. Moreover, expression of LPL solely in the heart is adequate to maintain normal levels of plasma triacylglycerol [52]. Pancreatic lipase gene has been identified that is preferentially or increasingly expressed in the heart of hibernating mammals [53]. Also, whole heart homogenate has shown moderately lipolytic activity in our investigation.

The principal purpose of the gas-filled swim bladder in fishes is to maintain a satisfactory state of buoyancy. A few fishes have swim bladders filled with lipid [54]. In the present study, none of these fishes contained any lipid material within their swim bladders, while tissue homogenate showed lipolytic activity that on the basis of tissue wet weight; its activity was higher than lipolytic activity of heart, stomach, gill and abdominal muscle. Patton and Thomas [55] have reported lipid-containing deposits within the swim bladders of *Coryphaenoides amolepis* and *Antimora rostrata*, they proposed lipase and lecithin:cholesterol acyltransferase action on the swim bladder lipids but they did not construct any enzymatic assays. We believe that swim bladders are partly dependent on lipid catabolism for their high energy demand. Further studies are needed to purify lipase in this organ. The concentration of the lipases in circulating blood is low, but they are rapidly released into blood by heparin and other polyanions [56]. LPL activity could not be detected in trout plasma before the injection of heparin. Lipase activity was present at a low level 8 minutes after injection and increased for approx. 35 minutes from the time of injection; it thereafter remained at an approximately constant value for a further 20 minutes [57]. We did not find any obvious difference between plasma lipase activities in comparison to 95° C heated plasma. Lipase activity was reported in oocytes of chicken [58], hawkmoth *Manduca sexta* [59] and cow [60] but we didn't detect it in rainbow trout gonads.

Fish spent large amounts of energy, particularly in osmoregulatory (gills, intestine and kidney) organs. Although, the functions of osmoregulatory organs have been extensively investigated, less attention has been paid to the metabolic aspects of osmoregulation in these organs [61]. In the present study, specific activity of lipase in kidney was not as high as in pyloric caecal mass and liver but higher than the specific activity that measured in other tissues. The LPL activity in the kidneys of several animal species such as mink, Chinese hamsters, rat and mice has been reported [62]. The method employed in this study to process lipolytic activity is not very specific to PTL therefore; the lipolytic activity may be due to LPL activity. To our knowledge, there are no comparable studies in the literature that mention to lipolytic activity in gills. Further studies are needed to in order to understand the role of lipase in gills.

CONCLUSION

A source of variation in lipase activity in different trout tissues might be related to the existence of different forms of this enzyme. Distribution of lipase in various extrapancreatic suggests that this enzyme may be functional in various physiological phenomena in the rainbow trout. It will be interesting to find the correlation between lipase activities with the level of mRNA of different forms of lipase to elucidate the pattern of lipases gene expression at the level of transcription. Future studies are needed to clarify involvement of lipase in various physiological processes and pathophysiological conditions in rainbow trout.

Acknowledgement

This work was conducted and funded by the Artemia and Aquatic Animals Research Institute, Urmia University, Urmia, Iran. The authors thank Dr Donovan German for his technical advices and critical reading of the manuscript.

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