A MOLECULAR VIEW OF LIPOPROTEIN LIPASE AND HEPATIC LIPASE STRUCTURE AND FUNCTION

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SUMMARY

Lipolytic enzymes play a pivotal role in the metabolism of triglyceride-rich lipoproteins circulating in plasma. The primary structures of many lipases have now been elucidated by molecular cloning of their cDNAs. To gain further insights into the molecular biology of lipoprotein lipase (LPL) and hepatic lipase (HL), we have determined their genomic organization. Both enzymes are members of a lipase gene family. Based on information derived from the genomic structures it is now possible to directly assess the molecular basis of familial LPL-defiency, a rare disorder of lipid metabolism involving the massive accumulation of chylomicrons in the plasma. Employing gene amplification techniques with LPL exon-specific oligonucleotide primers and direct DNA sequence determination, we have characterized a kindred with classical familial LPL-deficiency. Loss of LPL enzymatic activity was found to be caused by an amino acid substitution close to the putative active site.

INTRODUCTION

Lipolytic enzymes or lipases are catalysts in the absorption and incorporation of lipids into living organisms, as well as in the mobilization and endogenous transport of these water-insoluble molecules (Fig. 1). Among these enzymes, lipoprotein lipase (LPL) is vital for the metabolism and transformation of triglyceride-rich lipoproteins circulating in the plasma of humans and many animal species (1-4). This enzyme is synthesized by the parenchymal cells of numerous extrahepatic tissues and is transported to the luminal surface of vascular endothelial cells (5,6). In this location, LPL is the rate-limiting enzyme for the catabolism of circulating triglycerides associated with chylomicrons and very-low-density lipoproteins (VLDL). LPL enzymatic activity (7,8) and mass (9,10) can be measured after an intravenous injection of heparin, which displaces LPL from the endothelial cells. A hereditary disorder, familial LPL-

deficiency, is caused by structural aberrations in the LPL molecule resulting in accumulation of chylomicrons in the plasma of homozygous patients.

Another lipase, hepatic lipase (HL), is synthesized exclusively by hepatocytes and secreted to the sinusoidal space (11-14). HL seems to be involved in lipoprotein transformations. Specifically, HL activity has been associated with the conversion of HDL_2 to HDL_3, and therefore may be linked to the pathogenesis or prevention of atherosclerosis.

This report will focus on the most recent investigations of LPL and HL. Other lipolytic enzymes listed in Table 1 have been extensively reviewed (as a reference, see (15)).

ENZYMES	*SITES OF ACTION*
Lingual lipase	Lingual serous glands
Gastric lipase	Gastric mucosal cells
Pancreatic lipase	Pancreatic exocrine cells
Carboxyl ester lipase	Pancreatic exocrine cells
Lipoprotein lipase	Extrahepatic endothelial cells
Hepatic lipase	Hepatic endothelial cells
LCAT	Hepatic parenchymal cells
Hormone sensitive lipase	Adipocytes
Lysosomal acid lipase	Lysosomes

Table 1: Lipolytic enzymes involved in lipid absorption and metabolism.

LIPASE PRIMARY STRUCTURES

The primary structures of many lipases have recently been elucidated using cDNA cloning strategies and DNA sequence determination (16-28). Comparison of nucleotide and amino acid sequences has revealed areas of extensive homology between HL, LPL and pancreatic lipase (PL) (16,17,19,20). Fig. 1 shows pairwise comparisons of lipase amino acid sequences. Human LPL was chosen as an arbitrary reference sequence. Interestingly, lipoprotein lipases show the highest degree of amino acid conservation, ranging from 93% (human LPL vs. mouse LPL) to 73% (guinea pig LPL vs. chicken LPL). Analysis of evolutionary relationships has indicated that LPL is most highly conserved throughout evolution. HL and PL seem to have evolved at a much faster rate than LPL (17).

Sequence comparisons have shown a region surrounding serine-132 of human LPL to be particularly highly homologous in several mammalian species (Fig. 2). The structural significance for this high local sequence conservation most likely derives from its location at the catalytic center of the enzyme. This notion is supported by data derived from the recent crystallization and determination of the three-dimensional structure of human PL (26). The structure of hPL shows serine-152 which is homologous to hLPL 132 to be part of the Asp/His/Ser triad assumed to be crucial to enzymatic activity (26). Furthermore, studies utilizing site-directed mutagenesis have indicated that conservative amino acid alteration in the homologous position of rat HL drastically reduces catalytic activity (29).

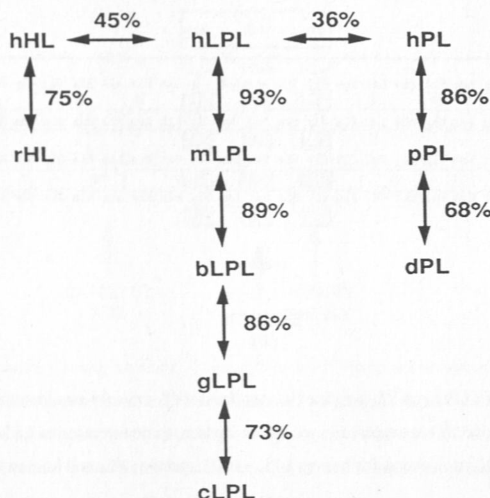

Fig. 1: Pairwise comparison of amino acid sequences of lipases. Numbers indicate percentages of identical amino acids based on maximum alignment sequence comparisons. Protein sequences were aligned using programs of the University of Wisconsin Genetics Computer Group Sequence Analysis Software Package. Abbreviations used are as follows: hHL, human hepatic lipase (16); rHL, rat hepatic lipase (19); hLPL, human lipoprotein lipase (20); mLPL, mouse lipoprotein lipase (22); bLPL, bovine lipoprotein lipase (23); gLPL, guinea pig lipoprotein lipase (24); cLPL, chicken lipoprotein lipase (25); hPL, human pancreatic lipase (26); pPL, porcine pancreatic lipase (27); and dPL, dog pancreatic lipase (28).

LIPASE GENE STRUCTURES

The striking amino acid sequence similarities of HL, LPL and PL strongly suggest that these genes evolved from a common ancestral gene. To investigate whether amino acid similarity is also reflected at the level of gene structure, we and others have elucidated the human HL and human LPL genomic organizations (30-33).

To elucidate the overall HL gene structure, genomic Southern blots were hybridized using ^{32}P- or digoxigenin-labeled cDNA fragments of HL as specific probes. For the detailed analysis of the HL gene, overlapping bacterial phage and cosmid clones spanning approximately 60 kb were isolated. Restriction mapping and hybridization analyses established the presence of 9 exons, ranging in size from 118 to 243 bp and separated by introns of 0.7 to >8 kb. The entire coding regions, the 5' flanking sequences, and the exon-intron boundaries were sequenced (31).

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                                  Gly - Ser - Gly
        Aa#
hLPL    123    Leu Asp Asn Val His Leu Leu Gly Tyr Ser Leu Gly Ala His Ala Ala Gly Ile Ala Gly Ser Leu Thr
rHL     138    Arg Ser Lys Val His Leu Ile Gly Tyr Ser Leu Gly Ala His Val Ser Gly Phe Ala Gly Ser Ser Met
pPL     143    Pro Ser Asn Val His Val Ile Gly His Ser Leu Gly Ser His Ala Ala Gly Glu Ala Gly Arg Arg Thr
hPL     143    Pro Ser Asn Val His Val Ile Gly His Ser Leu Gly Ala His Ala Ala Gly Glu Ala Gly Arg Arg Thr

                                             CATALYTIC
                                               SITE
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Fig. 2: Comparison of human LPL, rat HL, porcine PL, and human PL primary structures. The consensus sequence, glycine - x - serine - x - glycine, is encountered in all known lipases, some esterases and a large number of serine proteases (34). The structural information for human LPL, rat HL, porcine PL, and human PL are from Refs. (20), (19), (27), and (26).

The complete LPL gene spans about 30 kilobases (kB) and consists of 10 exons. The first exon encodes for a 27-amino acid signal peptide including the first two amino acids of the mature protein. Also, this exon encodes for 188 nucleotides of the 5' untranslated region. LPL exons 2 to 9 vary in length from 105 to 243 nucleotides and encode for the remaining amino acids of the lipase. Using primer extension and S1 nuclease protection assays, the LPL transcription start site was mapped. The 5' untranslated region contains regulatory elements similar to those found in many eucaryotic genes. The putative catalytic site for LPL is located in exon 4, whereas a postulated heparin binding site is present in exon 6 (34).

Interestingly, analysis of gene structures revealed a very similar gene organization for all three lipase genes (Fig. 3). For example, in human HL and human LPL, all exons with the exception of exons 2 and 8 are identical or very similar in length and the 5' and 3' ends are at nearly identical nucleotide positions. The PL gene organization differs from HL and LPL. The PL gene consists of 13 exons and 12 introns and thus contains five introns not present in HL. Intron loss due to recombination and sliding events during gene evolution may account for these differences. The extensive similarities of LPL, HL, and PL gene structures firmly establish them as members of a lipase gene family.

LIPOPROTEIN LIPASE DEFICIENCY

Familial LPL-deficiency (type I hyperlipoproteinemia) is a rare disorder of lipid metabolism characterized by a massive increase in chylomicron levels in fasting plasma and a resultant severe increase in plasma triglyceride levels (Table 2 and ref.(35)). This is associated with a variety of clinical features, including recurrent abdominal pain,

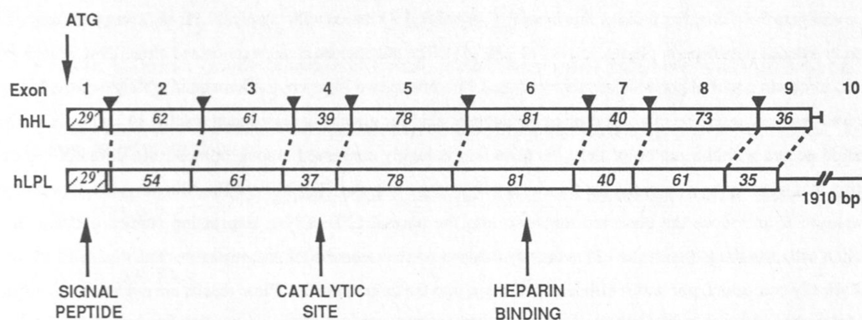

Fig. 3: Comparison of the human HL and human LPL gene structures. Exons are shown as boxes and intron positions as filled triangles. Hatched boxes, open boxes and lines indicate signal peptide, mature protein, and noncoding regions, respectively. Numbers in the open boxes indicate the exon length in amino acids. ATG refers to the translation initiation codon. Dashed lines between the two genes indicate location of exon-intron junctions. The structural information for human HL and human LPL genes are from Refs. (31) and (33).

hepatosplenomegaly, eruptive cutaneous xanthomas, and retinal lipemia. Detailed analysis of plasma lipoproteins reveals chylomicronemia alone or together with increased levels of VLDL in the fasted state. Triglyceride concentrations are usually above 17 mmol/l, with moderately elevated plasma cholesterol, and with decreased cholesterol levels in the low density lipoprotein (LDL) and high density lipoprotein (HDL) fractions. The disorder is usually, but not always, detected in infancy or childhood and is found in approximately 1 in 1 million in the population. It does not seem to predispose to atherosclerosis, but recurrent episodes of acute pancreatitis may threaten the patient's life (35-37). Dietary restriction of daily fat intake to 20 g or less or substitution of the diet with medium-chain triglycerides is required to control triglyceride levels and related clinical symtoms.

Familial LPL-deficiency has been extensively characterized by biochemical means (1-4,38). Reduction or absence of LPL activity in postheparin plasma or in adipose tissue establishes the diagnosis LPL deficiency. The cloning of the complementary DNA for human LPL (20,21) makes it possible to assess directly the molecular basis of familial LPL-deficiency. A recent study employing Southern DNA analysis indicated that gross alterations in the LPL gene are the cause of primary LPL deficiency in a significant number of type I hyperlipoproteinemias (Fig. 4, type I) (39,40).

Frequency	- rare
Mode of transmission	- autosomal recessive (McKusick #23860)
Clinical features	- manifested in childhood
	- chylomicronemia/ normal VLDL levels
	- reduced or absent activity of lipoprotein lipase
	- no premature atherosclerosis
	- therapy: fat restriction and medium chain triglycerides

Table 2: Clinical features of familial lipoprotein lipase deficiency.

To investigate the molecular basis of this disorder, we studied a kindred with classical LPL-deficiency as diagnosed by severely reduced post-heparin plasma activity of LPL (41). The polymerase chain reaction and direct DNA sequencing were used to obtain a partial genomic sequence of the LPL gene in two hyperchylomicronemic siblings. A single-base change was detected, resulting in a substitution of glutamic acid for glycine at amino acid residue 142 on exon 4. This substitution occurs within a region of the LPL gene that is highly conserved among lipases from different species, suggesting that this region is essential for the catalytic activity of lipases (Fig. 4, type IIa). Site-directed mutagenesis was employed to introduce the observed mutation into the normal LPL cDNA. Expression studies indicated that mammalian cells transfected with the LPL mutant produced normal amounts of enzyme mass. The mutated LPL was not catalytically competent, nor was it efficiently released into the culture media. These results are evidence that substitution of glutamic acid for glycine-142 has a major adverse effect on the tertiary structure of the aberrant LPL molecule leading to a functionally deficient protein. Thus, the properties of the expressed mutant LPL were found to be consistent with the LPL-deficient phenotype in the hyperchylomicronemic subjects (41).

TYPE	activity	mass / pre-hep	mass / post-hep	DEFECT
I	--	--	--	major genomic alteration
II a	--	--	+	mutation, deficient triolein hydrolysis
II b	--	--	+	mutation, deficient lipid binding
II c	--	--	+	mutation, deficient apo C-II binding
III	--	+	+	mutation, deficient heparin binding

Fig. 4: Molecular subclasses of lipoprotein lipase deficiency.

Other investigations of LPL-deficient patients have defined different point mutations in the LPL amino acid sequence (42,43). Based on this information, we surmise that considerable molecular heterogeneity is responsible for LPL deficiency. Perhaps, subtle changes of enzyme structure such as enzyme half-life or substrate affinity (Fig. 4, type IIb and ref. (44)) as well as interaction with heparin (Fig. 4, type III) and apolipoprotein C-II (Fig. 4, type IIc), are only slightly altering enzymatic function. Exploitation of the PCR technique in conjunction with DNA sequence analysis should facilitate the determination of structural variants of LPL. This will further aid in characterizing the role of LPL mutations in familial chylomicronaemia and related disorders of lipid metabolism.

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