thematic review

The lipase gene family

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Abstract Development of the lipase gene family spans the change in science that witnessed the birth of contemporary techniques of molecular biology. Amino acid sequencing of enzymes gave way to cDNA cloning and gene organization, augmented by in vitro expression systems and crystallization. This review traces the origins and highlights the functional significance of the lipase gene family, overlaid on the background of this technical revolution. The gene family initially consisted of three mammalian lipases [pancreatic lipase (PL), lipoprotein lipase, and hepatic lipase] based on amino acid sequence similarity and gene organization. Family size increased when several proteins were subsequently added based on amino acid homology, including PL-related proteins 1 and 2, phosphatidylserine phospholipase A1, and endothelial lipase. The physiological function of each of the members is discussed as well as the region responsible for lipase properties such as enzymatic activity, substrate binding, heparin binding, and cofactor interaction. Crystallization of several lipase gene family members established that the family belongs to a superfamily of enzymes, which includes esterases and thioesterases. This superfamily is related by tertiary structure, rather than amino acid sequence, and represents one of the most populous families found in nature.—Wong, H., and M. C. Schotz. **The lipase gene family. *J. Lipid. Res.* 2002.** 43: **993–999.**

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This thematic review is intended to serve as an introduction to a series of forthcoming review articles in the *Journal of Lipid Research* on several mammalian and avian lipases that are critical for the absorption and metabolism of lipids and lipoproteins. The articles will include: hepatic lipase, hormone-sensitive lipase, endothelial lipase, lipoprotein lipase, pancreatic lipase (PL), and bile salt-stimulated lipase.

ORIGIN OF THE LIPASE GENE FAMILY

Lipases are water-soluble enzymes that hydrolyze ester bonds of water-insoluble substrates such as triglycerides, phospholipids, and cholesteryl esters. Although lipases had been studied for over 150 years (1), the primary amino acid sequence of an entire lipase molecule was not accomplished until 1981 (2), when PL was sequenced. In 1986, several proteolytic peptides derived from lipoprotein lipase (LPL) were sequenced and shown to have amino acid homology to the known sequence of PL (3). A tryptic peptide from rat hepatic lipase was shown to be almost identical to a portion of bovine LPL (4). This led the authors to postulate that "these lipases form a multigenic family involved in the absorption and transport of lipid. Perhaps these enzymes arose from a common ancestral gene and diverged into distinct evolutionary pathways" (3). This speculation turned out to be correct, as an explosion of sequence and gene organization information on hepatic lipase and LPL from several laboratories reached the conclusion that LPL and hepatic lipase were part of a lipase gene family that included PL (5–7). Recent studies have implicated two other lipolytic enzymes to be closely related members of this lipase gene family, namely endothelial lipase (7–9) and phosphatidylserine phospholipase A1 (10, 11). In addition, two other members of the lipase gene family, i.e., PL-related proteins 1 and 2, have been identified and found to form a subfamily of PLs (12, 13). All members exhibit neutral or phospholipase activity, with the exception of PL-related protein 1 that, while normally inactive, can be induced to exhibit lipolytic activity with lipid substrates by the introduction of point mutations (14, 15).

FUNCTIONS

The physiological function for several members of the lipase gene family has been extensively studied. Pancreatic lipase is synthesized by pancreatic acinar cells where it is secreted into the intestinal lumen and aids in the intestinal absorption of long-chain triglyceride fatty acids (16, 17). Two proteins closely related to PL, PL-related proteins 1 and 2, have been found in the pancreas at lower

Abbreviations: LPL, lipoprotein lipase; PL, pancreatic lipase; single letter abbreviations for the amino acids are used.

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concentrations relative to PL (13). While PL-related protein 1 displays no enzymatic activity, it undergoes developmental regulation by exhibiting high neonatal mRNA levels that decrease in the adult pancreas (18). In contrast, PL-related protein 2 exhibits discordant developmental regulation compared with the other PLs and has hydrolytic activity against both triglyceride and phospholipid substrates, a feature distinguishing it from PL, which has a strict neutral lipid preference (19).

Other lipase members function in the metabolism of circulating lipoproteins. Hepatic lipase plays a role in the uptake of HDL cholesterol (20) and is synthesized exclusively in the liver, where it is predominantly found (21). A small fraction of total hepatic lipase protein is seen in the ovaries and adrenal gland and is proposed to attain that distribution by plasma transport and binding to tissue-specific sites (21, 22). A third member of the lipase gene family, LPL, is distributed in a variety of tissues, with the highest concentrations occurring in adipose tissue and muscle. This lipase is bound to capillary endothelium, where it functions to supply the underlying tissue with fatty acids derived from the triglyceride-rich core of circulating chylomicrons and VLDL (20). In the process, LPL transforms these lipoproteins into remnant and HDL particles. It should be noted that several groups have proposed that both LPL and hepatic lipase also have a non-enzymatic role as a ligand in the metabolism of plasma lipoproteins (23, 24). Under conditions of enzyme excess, they showed direct binding of hepatic lipase or LPL to cell surface receptors and lipoprotein particles resulting in cellular lipid accumulation that was independent of lipolysis. Nevertheless, under physiologically relevant conditions, it remains to be determined whether this non-enzymatic function contributes significantly to cellular lipid uptake.

A more recently discovered member of the lipase gene family, endothelial lipase, was cloned from endothelial cells but is expressed in a variety of tissues, including liver and thyroid. A complete review of endothelial lipase will be available in a future issue of this journal; however, though quite similar in amino acid sequence, the enzyme differs from hepatic and lipoprotein lipase in that it has a relatively high phospholipase activity compared with neutral lipid hydrolysis (7). This is even more true of another member of the lipase gene family, phosphatidylserine phospholipase A1, which has an absolute preference for phosphatidylserine and lysophosphatidylserine (10). The function of these two lipases is uncertain at this time, though endothelial lipase is believed to have a role in HDL metabolism (7–9), while phosphatidylserine phospholipase A1, produced by platelets, may act in response to apoptotic conditions by stimulating mast cell histamine release, thereby contributing to inflammation (25, 26).

PHYLOGENETIC RELATIONSHIPS

Sequence analysis has revealed phylogenetic relationships among the members of the lipase gene family. For example, based on a shared organization of intron-exon boundaries along with high sequence homology, it was proposed that PL, LPL, and hepatic lipase were derived from a common ancestor. It was also found that LPL and hepatic lipase were more closely related to each other than to PL (5, 6). Analyses of the full complement of the lipase gene family indicated that endothelial lipase is even more closely related to LPL than hepatic lipase and that phosphatidylserine phospholipase A1 diverged earlier from PL than LPL (7, 10).

In addition to direct sequence comparisons, the availability of the PL crystal structure and the ever-increasing protein structure database has led to the recognition of a superfamily that includes esterases and thioesterases (27). All members share a characteristic structural feature surrounding the active site residues called the α/β hydrolase fold (27, 28). This fold is responsible for maintaining the juxtaposition of conserved residues in the active site pentapeptide, and evolved independently from the forces that constrained and molded the analogous pentapeptide of serine proteases (29). Thus, despite shared sequence conservation, these two motifs most likely result from convergent, rather than divergent, evolution (30).

LIPASE GENE FAMILY SEQUENCE COMPARISONS

The possibility that one member would aid in determining the structure-function relationships for the entire family was not always realized. This is best exemplified by the difficulty in assigning a common active site serine among the family members. Early studies using emulsified diethyl *p*-nitrophenylphosphate to label PL identified a serine in a GXSXG pentapeptide sequence (where X = any amino acid) as a residue involved in interfacial binding, but not the putative active site of the enzyme (31, 32). Similar experiments of LPL treated with diisopropylfluorophosphate (DFP) yielded the conclusion that a labeled serine residue, in a GGS sequence, was the catalytic center of that enzyme (33). However, shortly thereafter, the complete LPL cDNA was made available and found not to contain a GGS sequence, thereby invalidating the labeling studies (34). The advent of the hepatic lipase cDNA did not aid active site identification, as that enzyme contained not only GGS, but also two GXSXG pentapeptide sequences. This GXSXG sequence was common not only to the three lipases but also to the known active center of serine proteases, prompting the suggestion that the lipase active serine was present in this sequence (29). The question was resolved experimentally when site-directed mutagenesis [first performed on hepatic lipase (35), then LPL (36), and finally PL (37)] conclusively identified the serine residue within the GXSXG sequence as the acylated center of the lipase gene family.

PL CRYSTAL STRUCTURE

The availability of the PL crystal structure provided important tertiary structure information that propelled in-

vestigations of the lipase gene family onto a different level. The PL structure was the first mammalian lipase to be solved and provided confirmatory, as well as novel, structural information compared with the structure of bacterial and fungal lipases. The amino-terminal domain of PL had striking similarities to that found in bacterial and fungal lipases, which are single-domain enzymes (38). However, PL also had a separate, discrete carboxyl-terminal domain that was absent in the other lipases (**Fig. 1**). This suggested that while the PL amino-terminal domain was conserved evolutionarily as the catalytic domain, a second domain evolved later to carry out important ancillary functions. Indeed, the high degree of amino acid sequence homology in the lipase gene family strongly suggests they share a similar structure that includes this distinctive carboxyl-terminal domain.

As seen in Fig. 1, the amino-terminal domain of PL is a series of nine β sheets arranged in a fan-like pattern, termed an α/β hydrolase fold. This fold is the defining characteristic of a large superfamily of proteins that includes esterases, lipases, and thioesterases (27, 28). These enzymes have the active-site serine residue that is centrally located in a pentapeptide sequence at a sharp turn between one end of an β strand and the start of the adjacent α helix, which has been designated "the nucleophile elbow" (27). The other two components of catalysis, typically aspartic acid and histidine, are also located nearby to form the catalytic triad of the enzymes.

In its inactivated state, the PL catalytic site is conspicuously inaccessible to substrate, being covered by surface loops that must move to accommodate a lipid substrate. The closed PL conformation (Fig. 1, bottom) converts into the open form (Fig. 1, top) upon interaction with lipid (39). The movement of the loops was initially thought to be the basis of "interfacial activation," a phenomenon that distinguishes lipases from esterases, and results in greatly increased activity in the presence of insoluble surfaces. It has been subsequently shown that interfacial activation is complex, possibly involving several different processes. Indeed, some studies have questioned whether lipase activation is even interfacial, based on attaining an activated ternary complex of PL, colipase, and a small micelle in the absence of any interface (40, 41). Despite these uncertainties, it is evident that the PL

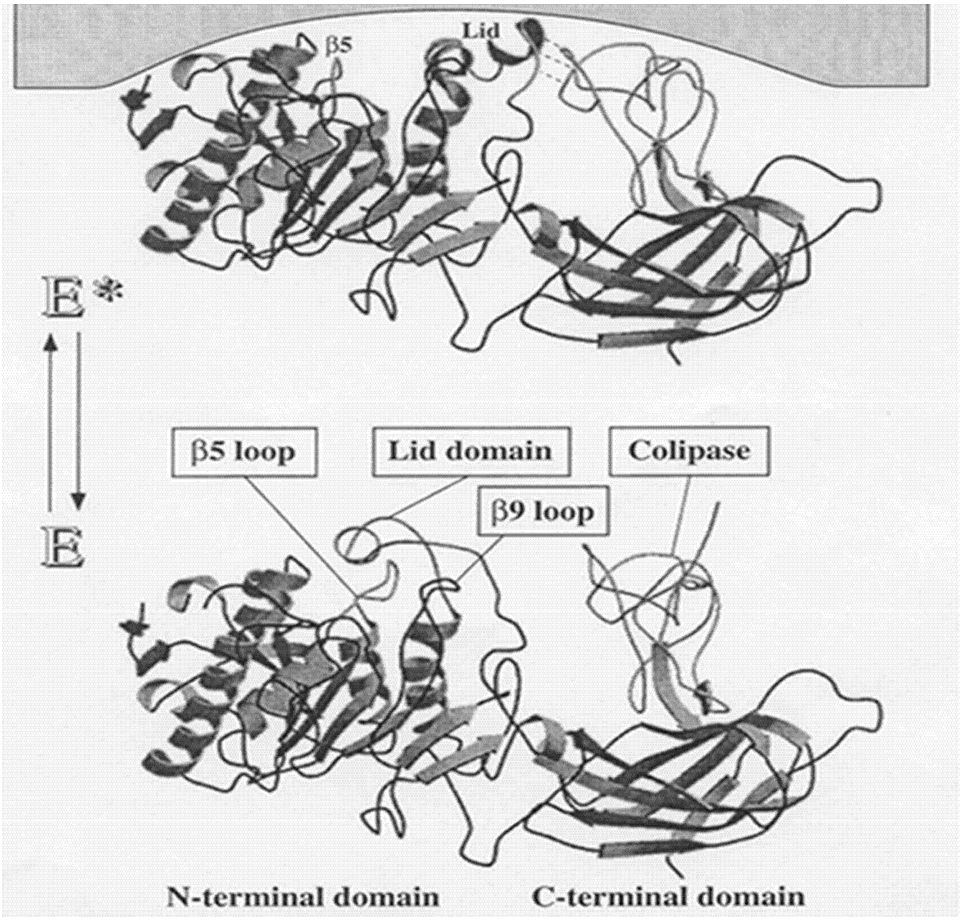

Fig. 1. The structure of human pancreatic lipase-procolipase complex in the closed (bottom) and open (top) conformations. Inactive enzyme (E) adapts the active form (E*) through interaction with micelles or lipid substrates, by movement of surface loops. The amino-terminal surface loops β5, β9, and the lid domain are indicated, as well as the primary colipase binding site on the carboxyl-terminal domain. This figure is reprinted from Miled et al. (63), copyright © 2000, with permission from Elsevier Science, and was adapted from van Tilbeurgh et al. (39, 64).

amino-terminal domain functions in the hydrolysis of ester bonds at the *sn*-1 position of triacylglycerols.

The carboxyl-terminal domain of PL is unique from its amino-terminal domain and features a distinctive series of β-sheets arranged in a sandwich configuration. In contrast to the catalytic machinery of the amino-terminal domain, this domain carries out important non-catalytic functions, such as colipase binding. Co-crystallization of colipase with PL has shown that this domain is the primary cofactor interaction site (39). The domain also binds micelles, and a surface loop, termed the lid domain (Fig. 1), derived from the amino-terminal domain that extends outward when in the open conformation. All of these functions are critical to physiological function of the enzyme. The binding of colipase alleviates bile salt inhibition by restoring enzyme binding to lipid substrates. Binding to micelles activates the enzyme to its open conformation, a process that involves interaction of the amino-terminal domain lid with both the PL carboxyl-terminal domain and colipase to expose a hydrophobic pocket and active site residues, thereby enabling substrate access (39). Other amino-terminal domain loop structures, such as β5 and β9 (Fig. 1), may also be required to move to allow full substrate entry (42), but a primary function of the PL carboxyl-terminal domain is to modulate substrate availability to the enzyme.

The crystal structure for members of the lipase gene family is currently limited to PL and its related proteins (15, 19, 42). Although structures for the other members have not yet been solved, computer modeling studies based on the PL backbone (43, 44) predict that hepatic lipase and LPL are also two-domain enzymes. This is not surprising because of the high degree of amino acid homology with PL (~50% in the amino terminal domains and ~30% in the carboxyl terminal domains) and the conservation in number and location of disulfide bonds. The LPL model has also been extrapolated to include the obligate dimeric structure of the enzyme and to explain functions unique to LPL (44). The veracity of such a model beyond the tertiary structure of the monomer is questionable, given that PL is not an oligomer and is in many ways functionally dissimilar from LPL. The absence of crystallographic information for lipases such as LPL limits the understanding of singular functional properties among lipase family members and has stimulated the use of alternative methods such as the domain-exchange strategy.

STRUCTURE-FUNCTION RELATIONSHIPS

The presence of two domains in PL has led to a better understanding of lipase structure-function relationships by development of the domain-exchange strategy (45). In this approach, domains or regions are exchanged between lipase gene family members and the functional parameters of the resultant chimeras determined. Generally, domain-specific functions are retained in the chimera, thereby disclosing the structural source of the function. The approach has been used with many members of the lipase gene family, yielding reliable findings because characteristic functions were retained in the chimeras, often to levels equivalent to that of the parental enzymes. The key to this approach is that independent regions from different enzymes retain their functional conformation due to the structural similarity shared among the lipase family members.

Although sequence similarities initially defined the lipase gene family, attention to functional differences between the lipases has produced a growing body of structure-function information. Thus, the terminal 136 amino acids of LPL retained properties similar to the native enzyme, in spite of being linked to the amino-terminal 329 amino acids of hepatic lipase (45). The chimera was fully functional as a lipase and, more importantly, displayed domain-specific properties characteristic of the parental enzymes. The regions responsible for specific functions were easily identified and candidate structures targeted for additional studies. Of equal importance was that the complementary chimera (the first 312 residues of LPL linked to the terminal 143 of hepatic lipase) provided correlative results, thus verifying the assignment of domain function (46). In this manner, the domain-exchange strategy has provided information on numerous important lipase functions, including substrate specificity, cofactor binding, and heparin binding (45–49). In addition, structure-function relationships have been investigated by an approach whereby lipases with altered primary structures were introduced into whole animals. These studies determined the in vivo effect of the changes and served to confirm in vitro observations (50, 51).

Coupled with other strategies, such as site-directed mutagenesis and truncation analysis, considerable information has been obtained regarding the lipase gene family. **Table 1** partially summarizes what is currently known about functional regions of the enzymes. The amino-terminal domains are generally catalytic in nature (for those capable of catalysis), containing the catalytic triad and, for LPL, a binding site for its cofactor, apolipoprotein (apo)C-II. The somewhat surprising finding that the PL cofactor, colipase, does not interact with the enzyme via the analogous domain can be explained by their dissimilar functions. Whereas colipase increases PL lipid affinity in the presence of bile salts, it does not directly facilitate catalysis (52). In contrast, apoC-II exerts its effects on reaction velocity and does not affect lipid affinity (53). In the presence of a lipid/water interface, colipase does interact with the PL through the lid region of the amino-terminal domain, but this occurs late in the activation process and presumably requires the presence of substrate or micelle (39). Thus, while both colipase and apoC-II are called cofactors, their effects are disparate and, not surprisingly, have distinctly different sites of interaction.

Certain lipase functions appear to require a complex interplay between domains. For example, the lid region, which is part of the amino-terminal domain, has been shown to influence substrate specificity (49), a parameter that other studies have shown to be affected by elements

TABLE 1. Domain location of structures and functions of the lipase gene family

	Amino-Terminal Domain	Carboxyl-Terminal Domain
Pancreatic lipase	Active site Surface loops and lid	Colipase binding Micelle binding Lid, in open conformation
Lipoprotein lipase	Active site Surface loops and lid ApoC-II binding Salt inhibition	Lipid binding Heparin binding ApoC-II binding Receptor binding
Hepatic lipase	Active site Surface loops and lid	Lipid binding Heparin binding Receptor binding
Pancreatic lipase-related protein 2	Active site Surface loops and lid	Colipase binding

in the carboxyl-terminal domain (46). ApoC-II binding to LPL may also require interaction with both domains to elicit full activation of lipase activity (48). For lipase gene family members known to be obligate dimers, such as hepatic lipase and LPL, the number of potential interactions expands greatly, and both intramolecular and intermolecular domain interplay are likely to define the unique functional characteristics of each enzyme.

Functions attributed to the carboxyl-terminal domain (Table 1) are generally binding or anchoring in nature. For hepatic lipase and LPL, two crucial enzyme functions, lipid binding (54–56) and heparin binding (45, 46, 57, 58), are influenced by residues in this domain. The lipid-binding residues affect the hydrolysis of insoluble substrates, despite the fact that the catalytic triad is buried within the amino-terminal domain. This suggests a sophisticated degree of communication between the two domains. For monomeric members of the lipase gene family, such as PL, this communication likely occurs intramolecularly. However, this communication may be intermolecular in the case of hepatic lipase and LPL, which are obligate dimers. It has been proposed that lipolysis requires interaction between the carboxyl-terminal lipid-binding elements of one subunit and the catalytic residues of the amino-terminal domain in the other subunit (59). Residues involved in heparin binding have also been attributed to reside in the carboxyl-terminal domain by chimera and site-directed mutagenesis studies (45, 46). For both hepatic lipase and LPL, heparin binding is used as a surrogate measure of binding to heparan sulfate proteoglycans, a physiologically important interaction that secures the lipase to capillary endothelium, providing access to circulating lipoproteins (20). In addition to heparin binding, studies have shown that residues within this domain direct binding of hepatic lipase and LPL to the LDL receptor-related protein, leading to cellular lipid accumulation (60–62). Finally, investigators have concluded that the LPL carboxyl-terminal domain contains elements important to maintaining the quaternary structure of the enzyme (56). Taken together, these findings support the modifying and accessory role of the carboxyl-terminal domain, complementing the catalytic functions performed by the amino-terminal domain of the lipase gene family.

SUMMARY

A measure of the significance of the lipase gene family is the importance of its members. The lipase gene family members are involved in a wide array of metabolic pathways, ranging from lipid digestion, absorption, fatty acid uptake, lipoprotein transformation, and inflammation. While much has been learned about the lipases, it is also clear that the complexity of this enzyme class extends far beyond our current understanding. There remains considerable uncertainty about basic processes like the mechanism of lipolysis and the numerous nuances of enzyme function that have yet to be solved. Due to the central importance of lipase function in lipid metabolism and transport, and its implication in serious diseases of the Western world such as obesity, diabetes, and atherosclerosis, it is imperative to know how lipases normally work. The lipase gene family has given us the opportunity to glimpse into those inner workings, and our view will continue to expand with the advent of new structural information.

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