Annu. Rev. Nutr. 1997. 17:141–58

STRUCTURE AND FUNCTION OF PANCREATIC LIPASE AND COLIPASE

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KEY WORDS: pancreas, protein structure, triglycerides

ABSTRACT

Dietary fats are essential for life and good health. Efficient absorption of dietary fats is dependent on the action of pancreatic triglyceride lipase. In the last few years, large advances have been made in describing the structure and lipolytic mechanism of human pancreatic triglyceride lipase and of colipase, another pancreatic protein that interacts with pancreatic triglyceride lipase and that is required for lipase activity in the duodenum. This review discusses the advances made in protein structure and in understanding the relationships of structure to function of pancreatic triglyceride lipase and colipase.

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0199-9885/97/0715-0141$08.00

INTRODUCTION

Triglyceride lipases are ubiquitous enzymes required for all aspects of fat metabolism (5). Lipases mediate the digestion of dietary fats, the uptake of fats into various tissues, and the mobilization of fats inside cells. In humans, triglyceride lipases are found in the gastrointestinal tract, bound to epithelial surfaces, and inside fat storage cells. Although some lipases will degrade a broad range of ester compounds, they all hydrolyze the ester bonds in acylglycerols, including the triglycerides that comprise greater than 95% of the dietary fats in the western diet (48).

The degradation of dietary triglycerides is critical for their utilization because triglycerides are not absorbed by intestinal enterocytes. Dietary triglycerides must be cleaved into free fatty acids and monoacylglycerols before they are absorbed (27, 28). In the absence of lipases, dietary triglycerides are not absorbed and pass in the stools. The resulting steatorrhea produces fluid losses, weight loss or poor growth, and deficiencies of fat-soluble vitamins.

In humans, the digestion of dietary triglycerides begins in the stomach, where gastric lipase releases about 15% of the fatty acids (6). Lipases, secreted by pancreatic acinar cells, complete fat digestion in the proximal small intestine. Of the known pancreatic lipases, pancreatic triglyceride lipase (PTL), the archetype of the lipase family, is clearly essential for the efficient digestion of dietary triglycerides. In patients with congenital absence of PTL, 50–60% of dietary fats are not absorbed (19, 21). This pivotal role in fat digestion has made PTL the subject of numerous investigations into its properties over this century. Despite this interest in PTL, relatively little was understood about the molecular basis for lipolysis until recent years, when the techniques of molecular biology and X-ray crystallography fostered a dramatic increase in our understanding about this unique class of enzymes. Previous reviews have discussed earlier work on PTL and on lipases from other species (3, 5, 8, 17, 25, 39, 57, 65, 69, 70). This review discusses recent advances, focusing on human PTL and its protein cofactor, colipase.

STRUCTURE OF PANCREATIC LIPASE AND COLIPASE

Primary Structure of Pancreatic Lipase

Human PTL was first isolated over 20 years ago by several different groups (20, 63, 66). Purified human PTL is a glycoprotein with an estimated molecular weight of 46,000. Over the years there were refinements in the isolation of human PTL, but the primary structure was not solved by chemical methods, as was accomplished for the pig PTL (11, 58). The primary structure of human PTL was finally predicted from the cDNA 20 years after its isolation (43). The

reported cDNA encoded a 465–amino acid protein of 51,156 Da. Comparison of the predicted sequence to the known N-terminal sequence of human PTL revealed that human PTL had a relatively short signal peptide of 16 amino acids. Cleavage of the signal peptide at the predicted site was confirmed by in vitro translation of the cDNA in the presence of microsomes, followed by amino acid sequencing of the [35S]methionine PTL. After removal of the signal peptide, the predicted size of the mature PTL was 449 amino acids, with a molecular weight of 49,558. A single N-linked glycosylation site was found at Asn140.

LIPASE GENE FAMILY The description of the cDNA encoding PTL and of the primary amino acid sequence strengthened the hypothesis that a family of lipolytic genes evolved from a gene encoding a common ancestral hydrolase. This hypothesis was proposed after alignment of the cDNAs encoding lipoprotein lipase and hepatic lipase showed marked homologies (35). Additionally, the proteins were of the same size, and alignment of their predicted amino acid sequences showed 30% and 33% identity of PTL with hepatic and lipoprotein lipase.

The subsequent characterization of the genes encoding these three lipases strengthened the hypothesis that they evolved from a common ancestor. Alignment of the exon-intron structure for the genes encoding human PTL, hepatic lipase, and lipoprotein lipase showed conservation of the exon-intron divisions, which indicated the genes are related (34, 56). Conservation of a repetitive sequence, an Alu element, in the corresponding introns of PTL and lipoprotein lipase increased the chances that the two genes are related (9, 56). Furthermore, classification of the Alu sequence suggested that the genes diverged within the last 40–50 million years.

The size of the lipase gene family was enlarged by the subsequent description of two pancreatic proteins with strong homology to PTL, the pancreatic lipase–related proteins 1 and 2 (PLRP1 and PLRP2) (22). The PLRPs were first identified in human pancreas, and one or both were identified in rat, mouse, dog, and coypu pancreas (24, 33, 51, 74). Human and rat PLRP1 have a short signal peptide, 17 amino acids, similar to PTL. The mature protein is slightly larger than PTL, 456 residues in rat and 451 in human PLRP1. Human and rat PLRP2 are also slightly larger than PTL, with 452 amino acids in the mature protein. Both have a 17–amino acid signal peptide. Comparisons of the available predicted amino acid sequences of PTL and the PLRPs reveal that the primary sequences are 63–68% identical and 77–81% homologous. The differences among these proteins demonstrate that they arise from separate genes, whereas the similarities imply that the genes evolved from a common ancestor of the lipase gene family.

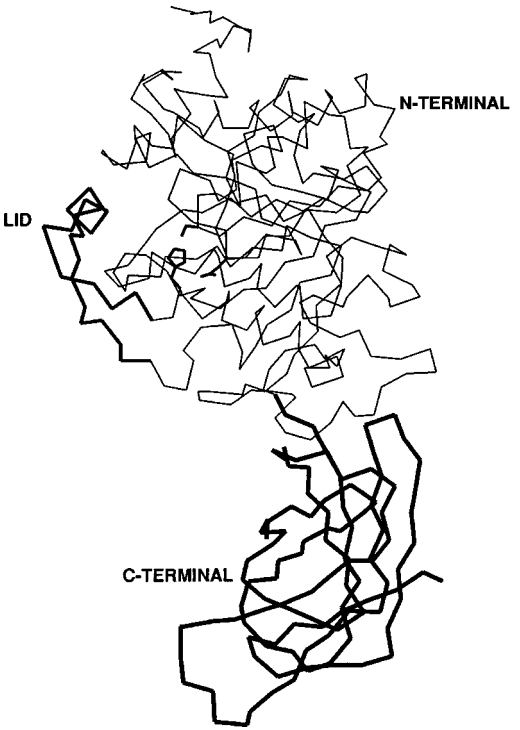

Figure 1 The tertiary structure of human PTL. The α-carbon backbone is shown. The C-terminal domain and the lid domain are in bold. The side chains of the catalytic triad are in bold.

Tertiary Structure of Pancreatic Lipase

The primary sequence data was supplemented by the tertiary structure of PTL, determined by radiographic crystallography at 2.8 Å (73). The structure showed that PTL is divided into two domains: a globular N-terminal domain formed by a central β-sheet core extending from amino acids 1–335, and a C-terminal domain consisting of a β-sheet sandwich structure (Figure 1). The domains are separated by a short, unstructured stretch of amino acids and are stabilized by seven disulfide bonds. The observed folding pattern of PTL is conserved in a number of hydrolases, including several fungal lipases (10). This finding further supports the concept of a lipase gene family.

Primary Structure of Colipase

The discovery, in 1910, that dilution of a glycerol extract from pancreas produced a precipitate and a filtrate that were inactive alone, but that had the

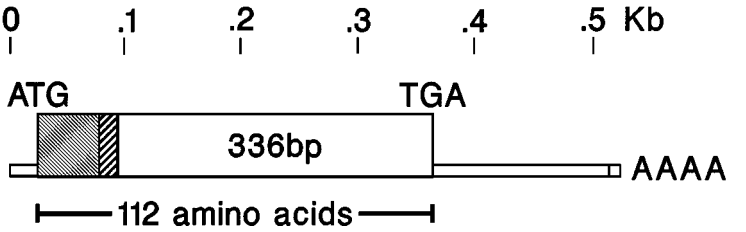

Figure 2 Schematic diagram of the human pancreatic colipase cDNA. The number of base pairs is given above the schematic. The signal peptide is indicated by the box with the thin diagonal lines. The propiece is represented by the box with the thick diagonal lines.

full PTL activity of the original extract when mixed, first suggested that PTL required another factor for activity (53). Over 50 years later, investigators separated PTL from a heat-stable factor by ion exchange chromatography and determined that the factor was a small-molecular-weight protein (1). Two groups described the ability of the cofactor to restore PTL activity in the presence of bile salts, providing an explanation for the paradox that purified PTL was inhibited by bile salts in vitro but was active in vivo with bile salts present (2, 46). The factor was partially purified and named colipase.

Sternby & Borgstrom purified human colipase in 1979, separating two isoforms of 10,000 daltons (59). The amino acid sequence, done by chemical methods, showed that colipase had 86 amino acids (61). Further studies found other forms of colipase. Colipase isolated from porcine pancreas contained an N-terminal pentapeptide that was absent from colipase isolated from porcine pancreatic juice (18). Assays of the two forms suggested that colipase isolated from pancreatic tissue was not as active as the form missing the N-terminal pentapeptide (72). Limited trypsin digestion of the tissue colipase released the pentapeptide, producing a colipase with the same N terminus and activity as colipase isolated from pancreatic juice (72). The hypothesis was proposed that colipase is secreted in an inactive form and activated in the duodenum by trypsin. This hypothesis accounts for the absence of a proform of PTL, as is found for many other digestive enzymes, and offers a mechanism to protect the pancreas and surrounding fat tissue from damage by PTL. For this reason, the tissue form of colipase was named procolipase.

A proform of human colipase was first identified in human pancreatic juice by amino acid sequence analysis of the N terminus (60). The cDNA for human colipase confirmed the presence of a propiece in human colipase and suggested that additional processing may occur at the C terminus (42). The cDNA is 525 bp, with an open reading frame encoding 112 amino acids with a calculated molecular mass of 11,595 (Figure 2). The first 17 amino acids form a signal

peptide and are followed by a 5–amino acid propiece. The protein sequence determined by chemical methods begins at amino acid 23 of the predicted sequence. From that point, the two sequences are identical until amino acid 91, where the sequence of the isolated protein ends and four additional amino acids are predicted from the cDNA. This comparison suggested that processing of human procolipase occurs at both the N and the C termini.

The importance of procolipase processing remains speculative. Although C terminus processing has been found in human and porcine colipase, there is no experimental support for a biological role of the cleavage. It may be incidental and a result of the high protease concentrations in the duodenum, or it may function to regulate the activity or concentration of colipase in the duodenum.

In contrast, several roles for the conversion of procolipase to colipase have been suggested by experimental data. As mentioned above, the decreased activity of procolipase compared with colipase at pH 8.0 led to speculation that the relatively inactive procolipase limited PTL in the pancreas (72). Several years ago that hypothesis was challenged by data showing that procolipase and colipase are equally active at neutral pH (36). Because the pH in these experiments was closer to the pH in the duodenum where procolipase functions, the authors suggested that the difference in activities at alkaline pH did not have physiological significance. The inactivity of procolipase at higher pH may not be a factor in the duodenum, but it may be important in the alkaline environment of the pancreatic duct.

A second possible function for the release of the propiece from procolipase was suggested by recent studies showing that the propiece pentapeptide decreased voluntary fat intake when given to rats (16). The pentapeptide was active if injected into the peritoneum or into the lateral ventricle. The last finding suggests that the peptide acts centrally. Furthermore, the peptide was found in the duodenum and in the circulation. This work led Erlanson-Albertsson to propose that the pentapeptide is a hormone, now called enterostatin, that regulates satiety (16). In this model, the release of enterostatin from procolipase by trypsin provides a feedback mechanism to the central nervous system, presumably to the hypothalamus, which limits dietary fat intake (16).

Tertiary Structure of Colipase

The presence of multiple forms of colipase may have hindered attempts to obtain crystals of colipase that provided good diffraction patterns. Whatever the explanation, the crystallization of procolipase proved elusive until it was crystallized as a complex with PTL (68). In this structure, procolipase is bound to the PTL C-terminal domain through two salt bridges involving Asp390 and Lys400 of PTL (Figure 3). Procolipase forms three major loops stabilized by

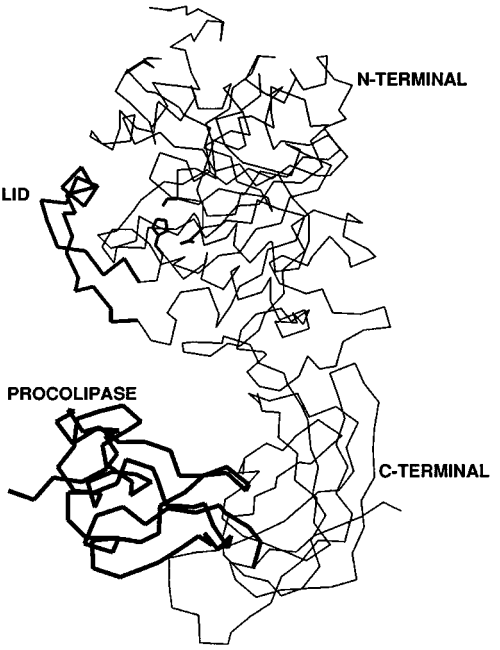

Figure 3 The tertiary structure of the procolipase-PTL complex. The α-carbon backbone is shown. Procolipase and the lid domain, in the closed position, are in bold lines.

disulfide bridges. The tips of the loops are on the surface opposite the PTL binding sites. This position and the predominance of hydrophobic residues suggest that these loops mediate interactions with the substrate interface. Procolipase did not form any interactions with the N-terminal domain and did not alter the conformation of PTL from that reported for the crystal structure of PTL alone.

MOLECULAR BASIS FOR PTL ACTIVITY

Catalytic Site

Solving the tertiary structure of PTL confirmed earlier suspicions that the enzymatic mechanism of PTL was similar to that of serine proteases. The inactivation of porcine PTL by esterification of a single serine implied a role for serine in the catalytic mechanism (70). The primary structure of PTL placed this serine in a short consensus sequence, G-X-S-X-G, shared by a number of lipases and esterases and felt to be located in the catalytic site in these enzymes

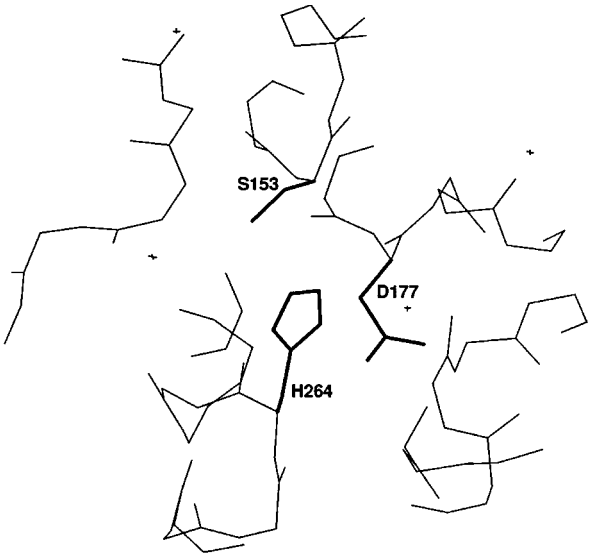

Figure 4 The catalytic triad of PTL. The α-carbon backbone is shown in thin lines. The side chains are in bold. The topology of the residues is similar to that seen in trypsin.

(43). This data, coupled with evidence that a histidine and acidic amino acid were required for the activity of porcine lipase, suggested the presence of the same Ser-His-acidic amino acid catalytic triad in lipases as is found in serine proteases (70). The crystal structure of human PTL located the conserved serine, Ser153, in the N-terminal domain, with its side chain in a hydrogen bond to His264, which in turn formed a hydrogen bond with Asp177. The topology of these three residues was nearly identical to that of the catalytic triad in trypsin, providing evidence that these residues formed a Ser-His-Asp catalytic triad in PTL (Figure 4).

Direct evidence for the function of these residues in catalysis was provided by site-specific mutagenesis of the cDNA encoding human PTL to produce recombinant proteins with mutations at each position (38). Replacing Ser153 with any of eight amino acids or changing His264 to leucine resulted in an inactive mutant PTL. In contrast, an Asp177 to glutamate mutant PTL retained 80% of its activity. This result differed from those reported for other enzymes, including lipases with a Ser-His-acidic amino acid catalytic triad. In those studies, mutating the acidic group from the native residue to another acidic amino acid greatly decreased the activity (14, 71). Thus, the preserved activity of the D177E mutant suggested that the assignment of Asp177 to the catalytic triad might be in error. Later, it was demonstrated that Asp206 could be rotated

into a position suitable for an interaction with His264, which supports the possibility that Asp206, not Asp177, functions in the catalytic triad (55).

Additional mutagenesis studies resolved this point. Mutant, recombinant PTL with Asp177 or Asp206 or both changed to alanine were tested (40). The D177A mutant had 20% of wild-type activity. The D206A mutant had normal activity, and the double mutant had only 2% residual activity. The combination of the crystal structure and these mutagenesis studies demonstrated that PTL requires a catalytic triad similar to the serine proteases and that Ser153, His264, and Asp177 comprise the triad. Interestingly, all three residues are conserved in the PLRPs, which suggests they may utilize the same catalytic mechanism as does PTL.

During these studies it was noted that the D177E mutant was degraded much more rapidly in the Sf9 medium than was wild-type PTL. In vitro studies showed that the mutant was also much more susceptible to trypsin. Obviously, the increased sensitivity to proteases is a disadvantage to a protein that functions in an environment rich in proteases. This finding provided an explanation for the preference of aspartate over glutamate in the active site of PTL, despite the ability of glutamate to effectively replace aspartate in the lipolytic reaction.

Interfacial Activation

Identification of the catalytic triad produced an unexpected finding in the tertiary structure of PTL (73). Several surface loops covered the active site and would sterically hinder binding of substrate to the active site. The largest of these loops, defined by a disulfide bridge between Cys238 and Cys262, was termed the lid domain. Two shorter loops—formed by residues 76–80, the β-5 loop, and 213–217—also blocked the active site. It was speculated that the movement of these loops would have to precede substrate binding.

The identification of these surface loops was particularly exciting because it provided a potential explanation for interfacial activation, a property found in enzymes, including PTL, that hydrolyze water-insoluble substrates (70). This property was described a number of years ago and was felt, until recently, to be a characteristic property of lipases (54). Interfacial activation refers to the large increase in activity observed for PTL when the substrate is particulate. PTL has activity against monomeric, water-insoluble substrates, but its activity increases greatly when the substrate forms water-insoluble particles such as micelles or emulsions (Figure 5). Speculation about the mechanism behind interfacial activation has focused on modifications of two models, an enzyme model and a substrate model (13). The substrate models proposed that the formation of substrate particles either increases the local concentration of substrate or alters the conformation of the substrate to permit hydrolysis. The enzyme models have generally included a conformational change in PTL, triggered by an oil-water

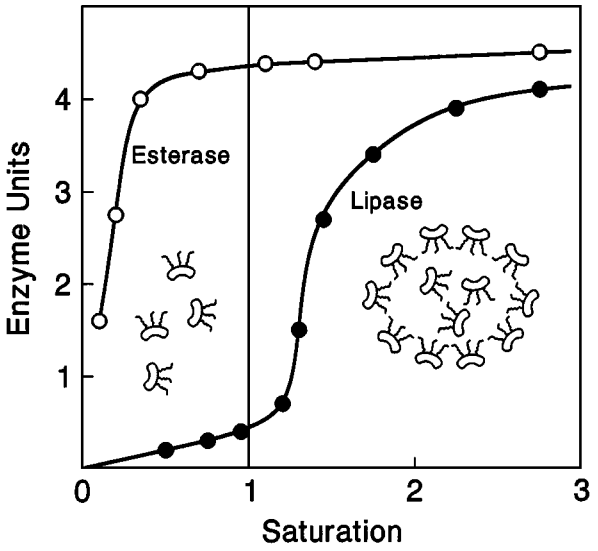

Figure 5 Interfacial activation of PTL. The activity of PTL and a hepatic esterase against triacetin are shown. The x-axis is the concentration of triacetin in multiples of saturation, the concentration when particles form. The symbols represent the physical state of the substrate, monomeric below 1 and aggregated above 1.

interface. The crystal structure of PTL suggests that a conformational change, movement of the lid domain and other surface loops away from the active site, is necessary to allow substrate into the active site, which lends support to the enzyme model of interfacial activation.

Further support for the enzyme model of interfacial activation has come from several different investigations. The first was a paper describing the second crystal structure of the colipase-PTL complex (67). In this study, crystals were obtained in the presence of octylglucoside and phospholipid mixed micelles. The observed structure showed several differences from the original structure of the colipase-PTL complex (Figure 6). There is a hinge movement of the C-terminal domain and of the lid domain. These two movements bring colipase into contact with residues of the lid domain. Importantly, movement of the lid domain is accompanied by changes in the β-5 loop, and together these two movements open and configure the active site to accept substrate. Concurrent studies on fungal lipases have also shown similar conformational changes in a surface loop homologous to the PTL lid domain (4, 12, 23). These studies clearly demonstrated the predicted conformational changes that were postulated to represent the physical correlates of interfacial activation and lend support to the enzyme model.

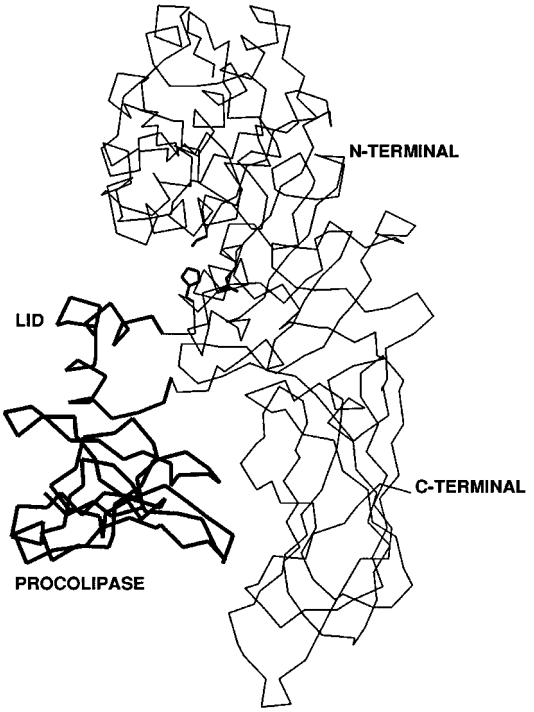

Figure 6 The tertiary structure of the procolipase-PTL complex in the presence of mixed micelles. The α-carbon backbone is shown. Procolipase and the lid domain, in the open position, are shown in bold. The side chains of the catalytic triad are also in bold.

Additional evidence that the opening of the lid domain correlated with interfacial activation was provided by the description of two PTL mutants (30). The cDNA of PTL was altered to create two deletions, one that removed the entire lid domain and another that removed the α-helix covering the active site. These mutants were expressed in a baculovirus system and the purified lipases were characterized. These mutants were active against substrate emulsions, but they were not activated by interfaces and could hydrolyze water-soluble substrates at a high rate. These results and the description of a guinea pig pancreatic lipase that does not have a lid domain and is not activated by interfaces support the enzyme model of interfacial activation and implicate the lid domain in the mechanism of interfacial activation (26).

Although movement of the lid domain appears to be critical to interfacial activation and for lipolysis to proceed, the presence of a lid domain does not insure that a lipase will possess interfacial activation. This observation arose

from studies on PLRP2 family members. Human, rat, and coypu PLRP2 have sequences homologous to the lid domain of PTL, which suggests that their active site should be covered (22, 51, 64, 74). Kinetic analysis of rat and coypu PLRP2 demonstrated that neither possess interfacial activation (32, 64). These results mean either that the PLRP2 lid does not occupy the same position in the tertiary structure or that the lid domain in the PLRP2 lipases is dynamic and spends a significant portion of time in the open position, whereas the lid in PTL prefers the closed position unless an oil-water interface is encountered. As a result, monomeric substrate is able to enter the active site of PLRP2, but it rarely encounters PTL with the lid in the open position.

The present evidence is weighted for the enzyme model of interfacial activation and against the substrate model, but lipolysis at oil-water interfaces may be influenced by both changes in the enzyme and in the substrate. Recent studies on lipid monolayers have demonstrated that lipids in the monolayer undergo structural changes during phase transitions induced by increasing surface pressures and that they have documented domain structures in monolayers that vary with temperature, pressure, and lipid composition (29, 52). All these physical changes in the monolayer can potentially alter lipase activity. Finally, it was recently shown that a lung surfactant protein, SP-B, had large effects on the formation of condensed phases in a monolayer and had large effects on a number of other physical properties (37). These findings suggest that the physical state of the substrate affects the quality of the interface, which in turn could influence lipolysis. Although additional studies showing that substrate influences lipases are needed to fully understand lipolysis, it now appears that the properties of the substrate and conformational changes in the protein may contribute to the ability of lipases to hydrolyze water-insoluble substrates at interfaces. These changes may not be exclusive but may be simultaneously induced in PTL and the substrate when the two come together in a complex, particularly when PTL is complexed with colipase, a small, surface active protein that may alter the physical properties of the substrate.

Colipase Function in Lipolysis

Interfacial activation may be a defining property of PTL, but the interaction of PTL with colipase may be the most important property of PTL because it is crucial for activity in the gut lumen (3, 17). PTL is inhibited by physiological concentrations of bile salts as well as by phospholipids and proteins that are present in emulsions of dietary lipids (Figure 7). Colipase restores activity to PTL under these conditions and permits the efficient digestion of dietary fats in the presence of multiple substances that inhibit PTL.

The mechanism of colipase reactivation has been speculative until recently. Most early models suggested that colipase binds to PTL and anchors PTL

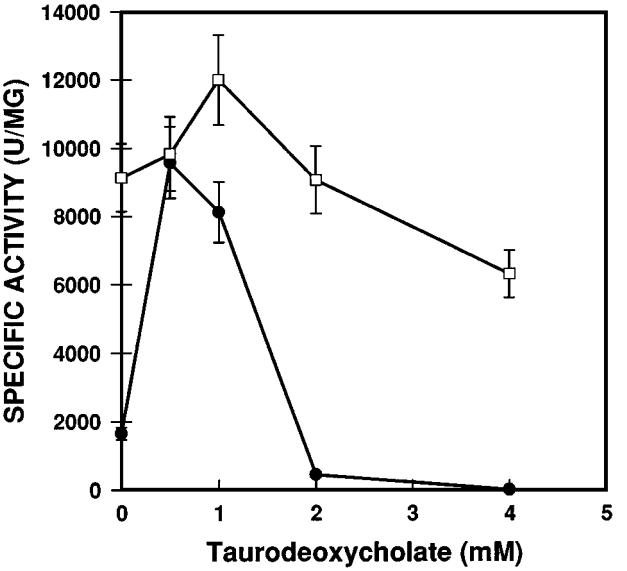

Figure 7 PTL activity in the presence of bile salt and colipase. PTL activity against tributyrin was assayed in a pH STAT with increasing concentrations of taurodeoxycholate. □ With added colipase; • no added colipase. The y-axis is specific activity, μmoles of fatty acid released per minute per milligram of PTL.

at the substrate surface, but other roles for colipase in lipolysis were seldom proposed (3, 17). Binding of colipase to PTL is supported by multiple studies utilizing a vast array of methods, including gel filtration, microcalorimetry, ultracentrifugation, binding of colipase to immobilized PTL, and phase partition techniques (7, 44, 49, 50, 62). Most evidence suggests that colipase binds to the C-terminal domain of PTL. For instance, the intact C-terminal domain isolated from porcine PTL after chymotrypsin digestion bound to colipase (7). Treating a mixture of pig colipase and horse PTL with a cross-linking agent resulted in cross-linking colipase to the C-terminal domain. The most convincing evidence of an interaction between colipase and the PTL C-terminal domain is the crystal structure showing the interaction of colipase and residues in the C-terminal domain (15).

Although much evidence supports an interaction of colipase with the C-terminal domain, a study of PTL mutants suggests that other interactions may occur as well. By introducing a stop codon in the cDNA of human PTL, only the N-terminal domain of PTL was expressed (31). This mutant had decreased activity against triglycerides, indicating that the C-terminal domain is required

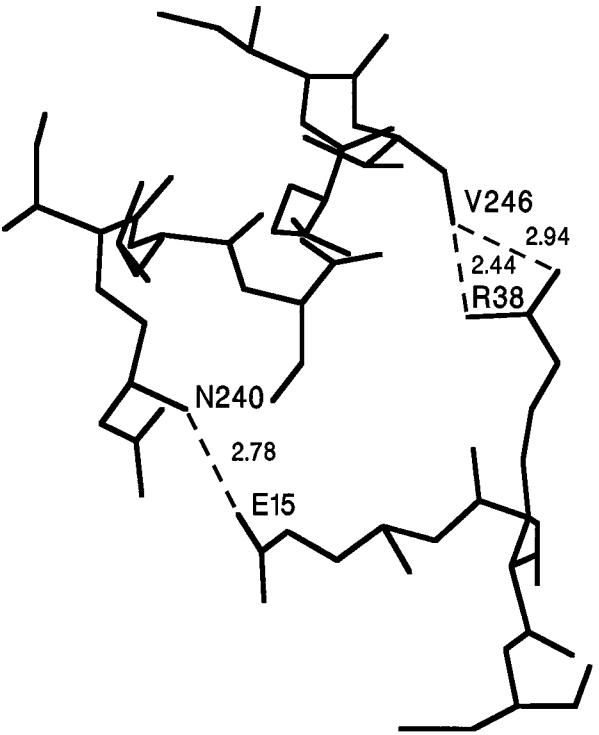

Figure 8 The contacts of colipase with the lid domain of PTL. The α-carbon backbone is shown. PTL is on top and colipase is below. The residues are labeled. The bonds are indicated by dashed lines and bond distances are given.

for full activity. Even so, the truncated PTL was still inhibited by bile salts. Activity was restored by colipase, which suggests that interactions with the N-terminal domain occur and are important for reactivation of bile-salt inhibited PTL.

Interactions between colipase and the N-terminal domain were demonstrated in the crystal structure of the colipase-PTL complex formed in the presence of mixed micelles (15). In this structure, hydrogen bonds formed between Glu15 of colipase and Asn241 and between Arg38 of colipase and Ser243 and Val246 (Figure 8). All the PTL residues are located in the lid domain, raising the possibility that colipase may function to stabilize the lid domain in the open position. Further evidence for this function was provided by analysis of a mutant colipase. Mutation of Glu15 in colipase decreased colipase activity 175-fold, but it did not interfere with the ability of the mutant colipase to anchor PTL to an interface (41). Thus, the ability of colipase to facilitate binding of PTL to

emulsions of triglycerides and bile salts and its ability to increase PTL activity against emulsions of triglycerides and bile salts were separated. This data is consistent with the hypothesis that colipase stabilizes the lid domain in the open position, thereby facilitating lipolysis.

MODEL OF PTL AND COLIPASE ACTION

Recent studies are providing insights into the molecular details of lipolysis that could not have been determined without the benefit of molecular biology and improvements in obtaining crystal structures of proteins. The mechanism of colipase and PTL still includes binding between the two proteins. This interaction could occur in the bulk phase or after colipase binds to the interface. Both pathways have been demonstrated experimentally. The interaction probably occurs initially between colipase and the C-terminal domain of PTL. But contact with the interface causes the lid domain of PTL to open, and new interactions are formed between colipase and PTL that increase the binding affinity of colipase, PTL, and the interface. It remains possible that interactions not observed in the crystal structures may be triggered by an oil-water interface. Once the lid is opened, the new interactions with colipase keep the lid in the open conformation, and lipolysis proceeds at a high rate catalyzed by the Ser-His-Asp triad.

Many questions about the mechanism of lipolysis remain. The details of bile-salt inhibition and the restoration of PTL activity by colipase are unclear. Studies of colipase binding to lipid monolayers suggest that colipase can cause lateral redistribution of the lipids in the monolayer (45, 47). Perhaps colipase creates patches of substrate and excludes inhibitory bile salts and phospholipids, allowing PTL to bind and function. Other questions about PTL function center on the active site. Where does the substrate bind? What determines substrate specificity and enantiomeric preferences? What determines stability at acid or alkaline pH? The information derived from these studies will lead to rational approaches for nutritional therapy for obese patients, for patients with pancreatic insufficiency, and for premature infants who have relative PTL deficiency.

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