thematic review

The triglyceride lipases of the pancreas

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Abstract **Pancreatic triglyceride lipase (PTL) and its protein cofactor, colipase, are required for efficient dietary triglyceride digestion. In addition to PTL, pancreatic acinar cells synthesize two pancreatic lipase related proteins (PLRP1 and PLRP2), which have a high degree of sequence and structural homology with PTL. PLRP1 has no known activity. PTL and PLRP2 differ in substrate specificity, behavior in bile salts and dependence on colipase. Each protein has a globular amino-terminal (N-terminal) domain, which contains the catalytic site for PTL and PLRP2, and a β-sandwich carboxyl-terminal (C-terminal) domain, which includes the predominant colipase-binding site for PTL. Inactive and active conformations of PTL have been described. They differ in the position of a surface loop, the lid domain, and of the β5-loop. In the inactive conformation, the lid covers the active site and, upon activation by bile salt micelles and colipase or by lipid-water interfaces, the lid moves dramatically to open and configure the active site. After the lid movement, PTL and colipase create a large hydrophobic plateau that can interact with the lipid-water interface. A hydrophobic surface loop in the C-terminal domain, the β5′ loop, may also contribute to the interfacial-binding domain of the PTL-colipase complex.—** Lowe, M. E. **The triglyceride lipases of the pancreas.** ***J. Lipid Res.*** **2002.** 43: **2007–2016.**

Supplementary key words triglyceride • X-ray crystal structure • C2-domain • colipase • site directed mutagenesis

The digestion and absorption of long-chain triglycerides, the major dietary lipid, is a highly efficient process involving several distinct steps, emulsification, hydrolysis by lipases into fatty acids and monoacylglycerols, dispersion of these products into an aqueous environment, and uptake by enterocytes (1). The first step, emulsification, begins with cooking, continues with chewing, and finishes in the stomach (1, 2). Several triglyceride lipases participate in the second step, the hydrolysis of dietary lipids. Hydrolysis starts in the stomach where, in humans, gastric lipase cleaves 15–20% of the fatty acids and goes to completion in the upper small intestine where the emulsion mixes with pancreatic juice containing several lipases capable of hydrolyzing triglycerides (3, 4). The resulting products form mixed micelles with bile salts, which, in turn, are absorbed by enterocytes.

The efficient digestion of dietary fats is all the more remarkable when the physical properties of the emulsion particles and the environment of the duodenum are considered (2, 5, 6). The emulsion particles form a complicated oil phase that creates an interface with the aqueous environment the lipases prefer. Virtually all of the dietary triglyceride and diglycerides segregate into the core of the emulsion particle, which is covered by a monolayer or multilamellar phase of mostly polar lipids, phospholipids, and fatty acids, with a small percentage of cholesterol and of triglycerides. Additionally, denatured dietary proteins, dietary oligosaccharides, and bile salts coat the surface, adding further complexity to the interface. As lipolysis proceeds, the composition and physical properties of the interface change continuously as products form and leave the interface (1). Consequently, the interface separates triglyceride lipases from the bulk of their substrate and presents obstacles to productive binding with interfacial substrate; that is, digestive lipases must associate with the scarce substrate in the interface and avoid nonproductive adsorption by the abundant amphipathic lipids.

The exocrine pancreas secretes several lipases that have overcome the kinetic challenges presented by emulsion particles. One of these lipases, pancreatic triglyceride lipase (PTL), predominates in vivo as evidenced by the fat malabsorption in patients with isolated PTL deficiency (7, 8). PTL belongs to the lipase gene family and is closely related to two other exocrine proteins, pancreatic lipase related proteins 1 and 2 (PLRP1 and PLRP2). Of these related proteins, only PLRP2 has triglyceride lipase activity in vitro (9). The role of the two related proteins in dietary fat digestion, particularly in adults, remains uncertain, although data in PLRP2 deficient mice indicate that this ho-

Abbreviations: C-terminal, carboxyl-terminal; 3-D, three dimensional; N-terminal, amino-terminal; PLRP1, pancreatic lipase related protein 1; PLRP2, pancreatic lipase related protein 2; PTL, pancreatic triglyceride lipase.

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molog contributes to dietary triglyceride digestion in the newborn (10).

Both PTL and PLRP2 have adapted to function at oil-water interfaces. Both lipases have much greater activity against water-insoluble substrates like long-chain triglycerides than they have against water-soluble substrates (11, 12). They both can efficiently find and associate with substrate in the interface, as evidenced by high rates of hydrolysis. PTL, which will not bind to interfaces containing bile salts, phospholipids, or proteins, overcomes the inhibition by binding to the interface in a complex with another exocrine protein, colipase (13). The contribution of colipase to PLRP2 function is less certain and may diverge among various species (9). In recent years, much has been learned about the molecular mechanisms that permit lipases to function at a hostile oil-water interface. This review will focus on the biochemistry and function of PTL and PLRP2. Necessarily, any discussion of PTL and PLRP2 must include colipase.

PHYSIOLOGY

Expression of PTL, PLRP1, and PLRP2 is mainly in the exocrine pancreas, but the amount of each lipase that is expressed varies from species to species and with age. PTL expression is found in most, if not all, adult vertebrates. In contrast, the expression of PLRP1 and PLRP2 varies greatly in the adult pancreas and their respective levels may be reciprocal. For instance, the pancreas of dog and cat contain equivalent amounts of PLRP1 and PTL, but do not have detectable levels of PLRP2 (14). Conversely, the horse pancreas has high levels of PLRP2 and no apparent PLRP1 (14). Guinea pig and coypu also possess high levels of PLRP2, but no information exists about the presence of PLRP1 (15, 16). Other species, like the rat, have small amounts of both PLRP1 and PLRP2 (17, 18). Small amounts of PLRP1 have been identified in human pancreatic secretions (19). In that study, PLRP2 was not detected in pancreatic secretions, but human pancreatic juice does contain a galactolipase activity that migrates with PTL on gel chromatography and that may represent PLRP2 since only PLRP2 has galactolipase activity (20). The situation in the newborn pancreas differs considerably, but only the rat, mouse, and human have been examined for mRNA expression and only one, the rat, for protein secretion (10, 21, 22). In these species, PLRP1 and PLRP2, but not PTL, are expressed prior to birth and expression persists into adulthood. In the rat and mouse, PTL is not expressed until close to weaning. A careful analysis of human newborns has not been done although they have low levels of lipase activity in pancreatic fluid up to 6–12 months of age, suggesting absent or low levels of PTL expression (23–26). Even though the analysis is incomplete, it is clear that these closely related genes show discoordinate temporal and species-specific expression. A better understanding of the molecular mechanisms behind the divergent expression may provide insight into the biological implications of the varied expression patterns.

SUBSTRATE SPECIFICITY

PTL is a carboxyl esterase with a strong preference for acylglycerides over phospholipids, cholesterol esters, and galactolipids (27, 28). A broad range of acyl chain lengths are cleaved from the α-position of triglycerides by PTL (29–31). The difference in the hydrolysis rates of long chain triglycerides (C_{14} to C_{22}) varies only 6-fold (31). Because PTL is secreted in large excess, these differences in rates are likely insignificant and all long-chain fatty acids should be efficiently cleaved by PTL. In addition to its role in triglyceride hydrolysis, PTL may contribute to the hydrolysis of retinyl esters in vivo (32). Extracts of mouse and rat pancreas contain a colipase-stimulated retinyl ester activity that eluted with PTL on ion exchange chromatography. In the same study, purified human PTL was shown to hydrolyze retinyl esters.

PLRP1 has little to no activity against triglycerides and no measurable activity against phospholipids, galactolipids, or cholesterol esters (17, 18, 21, 33). Based on comparisons of PTL and PLRP1 sequences and the crystal structure of dog PLRP1, two groups restored significant triglyceride lipase activity to PLRP1 by introducing two amino acid substitutions, V178A and A180P, into a putative acyl chain-binding site (18, 34). The mutant had easily demonstrable activity against tributyrin and trioctanoin.

These studies provide an explanation for why PLRP1 does not hydrolyze triglycerides, but they do not explain the physiological function of PLRP1 and why some species secrete large amounts of this lipase homologue. Amino acid sequence analysis of PLRP1 argues that the protein is not vestigial and that it probably has a physiological function. The conservation of the PLRP1 sequences among various species is as high as for PTL. Interestingly, the inactivating amino acid substitutions at positions 178 and 180 are conserved among all known PLRP1 proteins. Furthermore, the major structural elements have been preserved and most differences between PLRP1 and PTL arise in surface loops of the proteins, which indicates that the divergences between PTL and PLRP1 has not been random as would be expected for a vestigial protein.

PLRP2 has activity against a variety of substrates, but the substrate selectivity appears to depend on the species. All known PLRP2 lipases hydrolyze triglycerides although the specific activities vary among different species (12, 14–16, 33, 35). PLRP2 from rat, guinea pig, and coypu have activity against phospholipids, but horse PLRP2 had very low activity against egg yolk phospholipids. Interestingly, coypu and rat PLRP2 show a clear preference for phosphatidylethanolamine and phosphatidylglycerol over phosphotidylcholine, which is the predominant phospholipid in egg yolk (12). Horse PLRP2 was not tested against purified phospholipids and may yet show activity against other phospholipids (14). Guinea pig PLRP2 also prefers phosphatidylethanolamine and phosphatidylglycerol, but has more activity against phosphotidylcholine than rat or coypu PLRP2 (12). The guinea pig PLRP2 also has higher activity against galactolipids than do rat and coypu PLRP2 (12, 28). At present, the differences in substrate specificity among the various

PLRP2 lipases and between PLRP2 and PTL have not been correlated with differences in structure.

COLIPASE AND BILE SALTS

Efficient dietary fat digestion requires colipase and bile salts in addition to triglyceride lipases. The importance of bile salts in fat absorption is apparent when bile flow is absent. For instance, in humans with bile fistulas and greatly decreased duodenal bile acid concentrations, the intestine only absorbs about 50–75% of dietary lipids (36). Additionally, bile salts may alter the surface of the emulsion particles by clearing dietary proteins and oligosaccharides apart from their important role in fatty acid absorption. This action removes potential inhibitors of PTL from the oil-water interface. Although bile salts are not absolutely required for dietary lipid absorption, they are necessary for the nearly complete absorption of dietary fats that normally occurs.

Curiously, these same bile salts inhibit PTL activity in the duodenum (27). Many laboratories have demonstrated that colipase restores activity to bile-salt-inhibited PTL in vitro, but there is little data about the importance of colipase in vivo (**Fig. 1**). The congenital absence of colipase has only been reported once. In this report, the pancreatic juice from two brothers with steatorrhea had quite low colipase activity and their steatorrhea improved with the administration of pancreatic enzymes (37). A recent study of colipase-deficient mice confirmed the requirement for colipase in dietary fat digestion (38). The colipase-deficient mice had steatorrhea and undigested lipids in their feces, but only on a high fat diet. Wild type littermates had no steatorrhea on the same diet. On a low fat diet, the colipase-deficient mice had normal fat absorption, suggesting that other lipases can compensate for decreased PTL activity when fat intake is minimal. The results demonstrate that colipase has a critical, but not essential, role in the digestion of dietary lipids by PTL.

Neither bile salts nor colipase significantly activate PLRP1. Still, indirect measures indicate that PLRP1 interacts with colipase (17). Solid phase assays show that PLRP1 binds to colipase that has been immobilized on membranes. Kinetic studies also support binding between colipase and PLRP1. In these experiments, PLRP1 inhibited PTL activity in the presence of colipase and bile salts. Increasing the colipase concentration restored activity, suggesting that PLRP1 competes with PTL for colipase. More convincingly, colipase restored activity to a chimera of the PTL amino-terminal (N-terminal) domain and the PLRP1 carboxyl-terminal (C-terminal) domain, the colipase-binding domain in PTL (see below), when bile salt micelles were included in the assay. At present, no data addresses whether or not PLRP1 binds colipase in the duodenum. If it does, then PRLP1 may modulate PTL activity in species, like the dog, that secrete large amounts of PLRP1.

The effects of bile salts and colipase on the activity of PLRP2 vary among different species. Human, mouse, and rat PLRP2 maintain activity in the presence of micellar concentrations of bile salts (33, 39, 40). Colipase increases activity for all three PLRP2 lipases. The increases range from 1.5- to 5-fold for rat PLRP2, the best studied of the three lipases (40). On the other hand, the horse, coypu, and guinea pig PLRP2 lipases are inhibited by micellar concentrations of bile salts and colipase does not restore activity to these PLRP2 lipases (14–16). A chimera of the guinea pig PLRP2 C-terminal domain and the human PTL N-terminal domain produced a recombinant lipase that is poorly activated by colipase in the presence of bile salts (41). The result suggests that the impaired colipase activation of guinea pig PLRP2 results from weak binding of colipase to this lipase. The differences in kinetic properties of the various PLRP2 lipases imply that these proteins may represent emerging branches of the lipase gene family and that they should not be grouped together. Whether the differences represent physiologically important changes in the enzymes or are inconsequential cannot be determined until the function of these lipases in neonates and adults of multiple species is better defined.

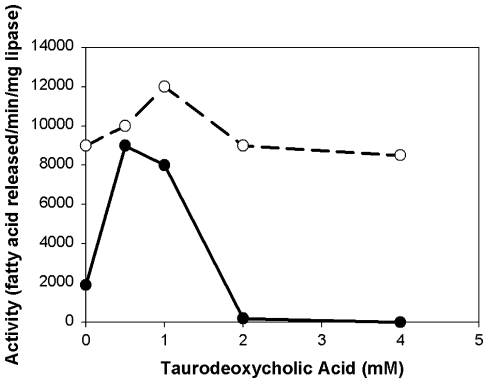

Fig. 1. The effects of bile salts and colipase on the activity of human pancreatic triglyceride lipase (PTL). PTL activity was measured in a pH-stat titrator with tributyrin emulsified with various concentrations of taurodeoxycholic acid. One and four tenths micrograms of recombinant human PTL was included in the assay. A 5-fold molar excess of recombinant human colipase was added as indicated. Closed circles, no colipase; open circles, with colipase.

LIPASE STRUCTURE AND FUNCTION

Our understanding of pancreatic lipase structure and function has increased tremendously through a series of three dimensional (3-D) structures of pancreatic lipases. The first crystal structure was that of human PTL (42). This structure was quickly followed by the determination of the human PTL-porcine colipase complex in two different orientations, of horse PTL, and the porcine PTL-colipase complex (43–47). Finally, 3-D structures of canine PLRP1 and rat PLRP2 have been reported (12, 18). Not only do these proteins have high primary sequence homology, their 3-D structures can be superimposed on that of human PTL (**Fig. 2**). Like human PTL, PLRP1 and PLRP2 have two distinct domains, an N-terminal domain

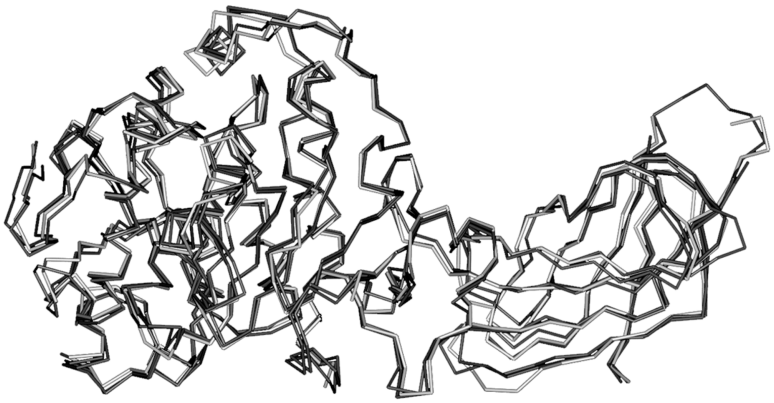

Fig. 2. The α-carbon trace of human PTL superimposed on the traces dog PLRP1 and rat PLRP2. Black, human PTL; Light gray, dog PLRP1; Dark gray, rat PLRP2. The coordinates were obtained from the Protein Data Bank and the figures were prepared with the MOLMOL program (75).

from residue 1 to 336 and a C-terminal domain from residues 337–449.

N-terminal domain

The N-terminal domain has a structure, the α/β hydrolase fold, which is present in other lipases and esterases (48). By analogy to serine proteases, Winkler et al. proposed that N-terminal domain residues Ser 153, Asp177, and His 263 formed the catalytic site. Subsequently, this assignment was confirmed by site-specific mutagenesis of these residues (49, 50). These residues are conserved in all known PTL, PLRP1, and PLRP2 lipases, and the structure around the catalytic serine has only been found in lipases and esterases.

Lid domain

In the human PTL structure, a surface loop defined by a disulfide bridge between Cys238 and Cys262, covers the catalytic site. This loop, named the lid domain, forms van der Waals contacts with the β5 loop containing residues 76–85 and the β9 loop of residues 204–224. Together the lid domain and the two loops sterically hinder access of substrate to the active site (**Fig. 3**). In the present conformation, PTL would necessarily be inactive and another conformation with an exposed active site was predicted (42).

A second, active conformation was subsequently identified in studies of the 3-D conformation of the human PTL-porcine colipase complex (Fig. 3). In these studies, the investigators obtained crystals in the presence and absence of octylglucoside and phospholipid mixed micelles (43, 44). Without micelles, the lid domain remained in the same closed position as observed in the human PTL structure. With micelles in the crystallization solution, the lid domain and the β5 loop adopt new conformations. A 29 Å hinge movement of the lid domain moves this region

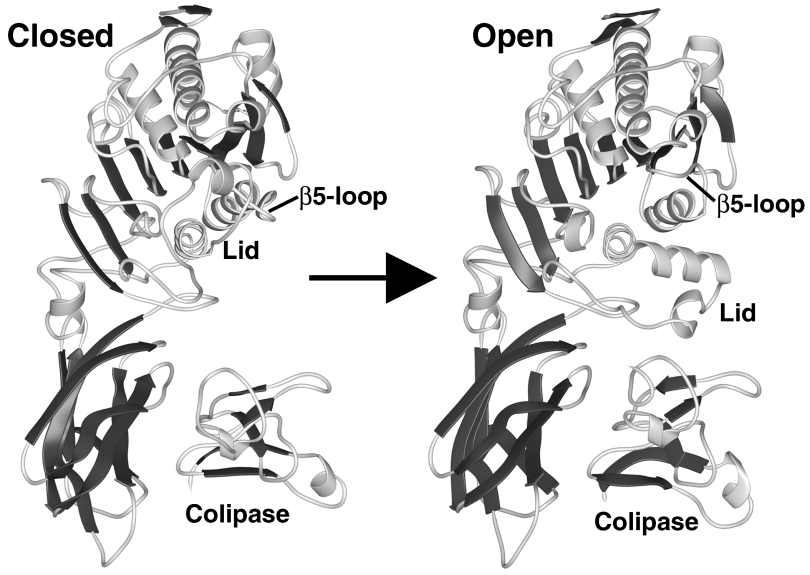

Fig. 3. Crystal structure of the PTL-colipase complex in two different conformations. The α-carbon trace is presented as a ribbon diagram where the arrows are β-strands and the thick ribbons represent α-helices. The globular amino-terminal (N-terminal) domain contains the lid and the β5-loop. The β9 loop is not marked. Colipase binds to the carboxyl-terminal (C-terminal) domain. The coordinates were obtained from the Protein Data Bank and the figures were prepared with the MOLMOL program (75).

away from the active site and the β5 loop folds away from the catalytic site. The β9 loop position remains unchanged. Together these movements open the catalytic site and reconfigure the region to create the oxyanion hole, an electrophilic region formed by Phe78 and Leu 154 that stabilizes the transition state intermediates. Additionally, the movements organize the acyl chain binding sites as demonstrated in studies of the PTL-colipase complexes with one or the other enantiomer of a C11 alkylphosphonate inhibitor in the active site (51). Acyl binding sites were identified on the β9 loop and on the open lid domain.

The contribution of the lid domain to substrate specificity has been further defined by site-directed mutagenesis. This technique was used to exchange the lid domains between PTL and PLRP2 lipases. In one series of studies, the lid domains of the guinea pig PLRP2, which has a naturally occurring lid truncation, and of human PTL were exchanged (41, 52). The PTL mutant had 10-fold lower specific activity against triglyceride substrates, but still did not cleave phospholipids. In contrast, the addition of the PTL lid to guinea pig PLRP2 decreased phospholipase activity disproportionately to the mutant's relatively preserved triglyceride lipase activity. The ratio of phospholipase to lipase activity was reduced to 0.07 when the PTL lid was added to PLRP2 compared to 0.34 for the native PLRP2 (41). This study suggests that the lid domain contributes to substrate selectivity, and shows that the size of the lid alone does not determine if a lipase is also a phospholipase.

Another group reported lid domain exchanges between rat PTL and rat PLRP2 (53). In this case, the lid substitutions affected the activity against various triglycerides disproportionately. The presence of the PLRP2 lid on PTL caused a 300-fold decrease in activity against tripropionin, a 20-fold decrease against triolein, a 10-fold decrease against tributyrin, and an 8-fold decrease against trioctanoin. The effect of putting the PTL lid on PLRP2 was much smaller, but did result in an increase in activity against tripropionin, trioctanoin, and triolein with a small decrease in activity against tributyrin. These results show that residues in the lid play a significant role in determining substrate preference for both PTL and PLRP2. The greater effect of the lid domain exchanges on PTL suggests that the lid domain plays a greater role in substrate specificity for PTL than for PLRP2. Of further note, the phospholipase activity of PLRP2 was unaffected by the lid exchange, affirming the notion that it is not the lid alone that determines whether or not a given lipase also has phospholipase activity.

Lipid-binding domain

A second consequence of lid domain and β5-fold movement is the creation of a hydrophobic plateau (**Fig. 4**). Comparison of the surface properties of the two different PTL-colipase complexes reveals a marked difference in exposed hydrophobic surface. With the conformational change of PTL, the open lid and the surface of colipase form a continuous hydrophobic surface extending over

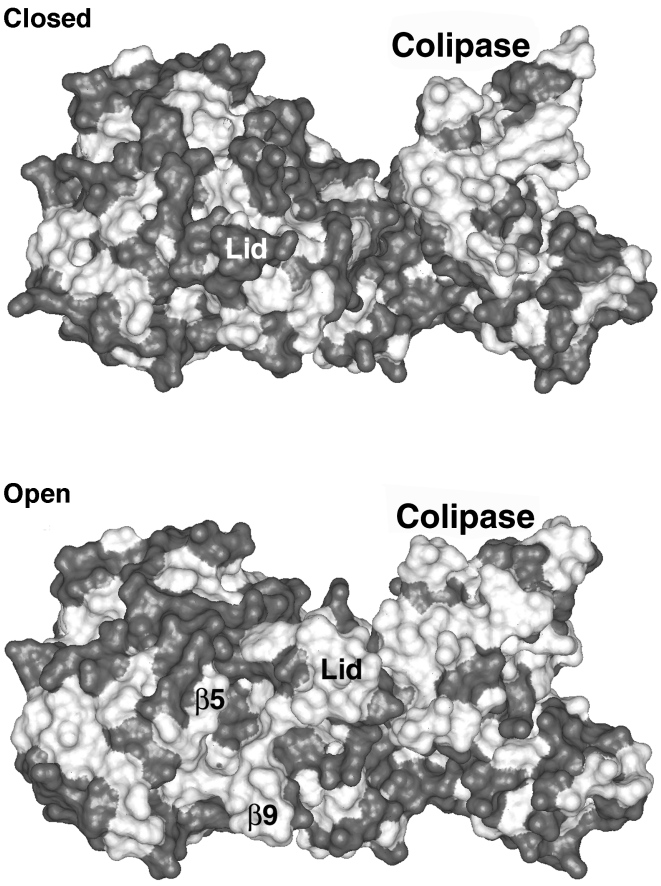

Fig. 4. The surface representation of the PTL-colipase complex in the open and closed conformations. Hydrophobic residues are light shading. Other residues are dark shading. The lid, β5, and β9 loops form a large hydrophobic plateau. The coordinates were obtained from the Protein Data Bank and the figures were prepared with the MOLMOL program (75).

50 Å. This surface was predicted to interact with the lipid-water interface.

Once again, site-directed mutagenesis studies addressed the role of the lid domain in interactions with an interface. In one approach, the lid domain was truncated by removing the α-helix that covers the active site (residues 247–258) or removed altogether (residues 240–260) (54). Both mutants retained activity against tributyrin and triolein, but their specific activity against both substrates was greatly reduced. The reduction in activity was explained in part by decreased adsorption of the lid mutants to triglyceride-bile salt emulsions. The long lag phase observed with triolein probably represents decreased binding of the lid domain mutants to triolein-bile salt emulsions. Direct measurements of adsorption to tributyrin-bile salt emulsions, directly demonstrated decreased adsorption of the lid mutants to this lipid-water interface.

While the above studies supported the role of the lid domain in binding of the PTL-colipase complex at the lipid-water interface, another study questioned the importance of the lid to lipid binding of the PTL-colipase complex (41). These investigators measured the binding to trioctanoin of a human PTL chimera with the truncated lid

domain of guinea pig PLRP2 and found no significant differences between the chimera and native PTL. The chimera bound to trioctanoin emulsions to the same extent as native PTL. The explanation for the opposite conclusions between these two studies may lie in the technical differences. Obviously, different triglycerides were tested in the two studies and the lipid-water interfaces may not be physically equivalent. Perhaps, more importantly, the times allowed for binding were considerably different. The first study measured binding to tributyrin over several minutes, whereas the second study incubated the lipases with trioctanoin for 1 hour. The long incubation may obscure small but significant differences in affinity. Resolving this question will require more careful titrations of binding isotherms and detailed studies of the orientation and conformation of the PTL-colipase complex at the interface.

The trigger for the movement of the lid domain remains speculative. Most have assumed that an oil-water interface triggers the conformation change in PTL (11). However, several studies have demonstrated that the open conformation can exist in the absence of an oil-water interface. PTL inhibition by tetrahydrolipstatin or by di-isopropyl p-nitrophenylphosphate requires the presence of bile salt micelles and colipase (47, 55, 56). The implication of these results is that bile salt micelles and colipase caused the lid to open so that the inhibitor could reach the active site. Another group showed that a monoclonal antibody, which only recognizes the closed conformation of human PTL, did not bind to PTL in the presence of bile salt micelles or water-miscible organic solvents (57). They interpreted these results to mean that bile salts micelles alone can trigger lid opening. Lipid-water interfaces were not present in any of these experimental systems proving that the PTL lid can open in the absence of substrate aggregates. Bile salt micelles with or with out colipase may be sufficient to open PTL even in the aqueous phase.

Ternary complex

Recent articles support the existence of a ternary PTL-colipase-bile salt micelle complex in solution (47, 56, 58). Low-resolution neutron diffraction of porcine PTL-colipase-detergent crystals identified a distorted disk-shaped detergent micelle in contact with colipase and the C-terminal domain of PTL (47). The same group followed this study with another that utilized small angle neutron scattering to demonstrate the formation of a PTL-colipase-taurodeoxycholate micelle complex in solution (56). Furthermore, they show that the ability of micelles to activate PTL correlates to their size and that mixed micelles of bile salt and fatty acids or lysolecithin activate PTL more efficiently than micelles of bile salts or phospholipids alone. They conclude that the mixed micelles in the duodenum are well suited to activate PTL and that the formation of a ternary PTL-colipase-mixed micelle complex determines the adsorption of the PTL-colipase complex to the lipid-water interface.

Several older studies strengthen the notion that PTL and colipase form a complex with constituents of bile prior to adsorption to the emulsion surface (59–62). When colipase and PTL are incubated with bile, they tightly associate with heteroaggregates of phospholipids, cholesterol, bile salts, and a biliary protein (61, 62). In vitro, these complexes are driven toward the lipid-water interface (62). These findings led to a proposed model of PTL-colipase complex formation in vivo. In this model, the mixing of bile and pancreatic juice occurs in the common channel, which is formed by the juncture of the bile duct and main pancreatic duct, making it possible for PTL-colipase-heteroaggregate complexes to form prior to secretion into the duodenum. The preformed complex could then be directed toward the dietary lipid emulsions where the PTL-colipase complex can adsorb to the surface. Two possibilities exist for the fate of the biliary lipids. They may remain as a heteroaggregate with the complex or the components may dissociate from the complex and spread onto the lipid-water interface (1).

Once the lid domain opens, several new interactions stabilize the new conformation (43). Hydrogen bonds form between Glu15 of colipase and Asn241 of PTL and between Arg38 of colipase and Val246 of PTL (**Fig. 5A**). Substitution mutagenesis of Glu15 in colipase decreased colipase activity 175-fold, but did not interfere with the ability of the mutant colipase to anchor PTL to a lipid-water interface (63). Thus, the ability of colipase to facilitate binding of PTL to an interface was separated from a distinct effect of colipase on PTL activity. The data suggests that colipase stabilizes the lid domain of PTL in an open and active conformation, thereby facilitating lipolysis.

A second set of interactions occurs between residues in the lid domain and in the core of PTL. When the lid opens, Arg257 and Asp258 in the lid form salt bridges with two residues, Tyr268 and Lys269, in the core of the protein (43). Interestingly, none of these residues are conserved in PLRP2 lipases (14, 53). Two studies have examined the role of these residues in PTL function by introducing either single or multiple mutations at these sites (52, 53). Either mutating the two lid residues to the corresponding PLRP2 residues or mutating all four residues to the corresponding PLRP2 residues resulted in decreased activity against triglycerides at submicellar concentrations and loss of activity at micellar concentrations of taurodeoxycholate. Single substitution mutations at Arg257 or Asp258 decreased activity against triglycerides in submicellar concentrations of taurodeoxycholate, but only mutations at Arg257 abolished activity in micellar concentrations of taurodeoxycholate. All but one of the Asp258 mutations retained 40% to 100% of activity depending on the amino acid substitution and on the substrate. For these mutants, activity against trioctanoin was preserved whereas activity against tributyrin and triolein was decreased. The exception was D258R, which had only 10–20% of normal activity. Clearly, the open lid requires the interactions of Arg257 and Asp258 with the core residues to achieve an active conformation. Any disruption of the interactions prevents the lid from attaining an optimal conformation.

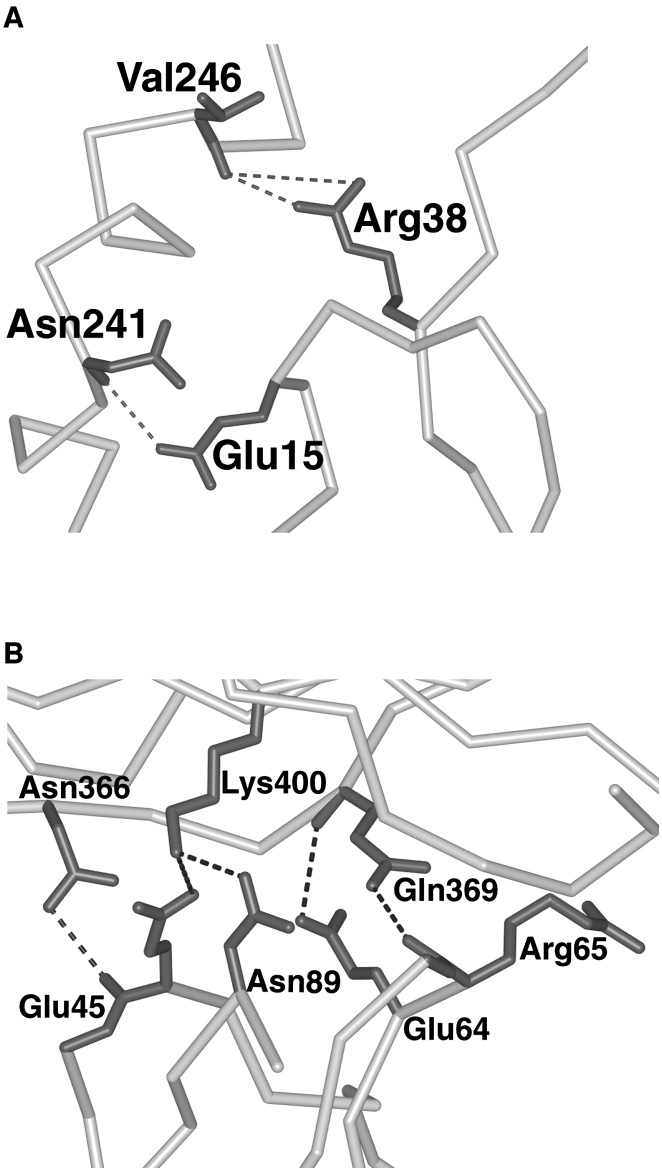

Fig. 5. Contacts between colipase and PTL. A: Colipase and the PTL lid domain. The side chains of the interacting residues between are shown in dark gray. The α-carbon trace in the region is shown in light gray. The interacting atoms are connected by dotted lines. Glu15 and Arg38 are colipase residues. Asn241 and Val246 are residues in the PTL lid domain. B: Colipase and the C-terminal domain of PTL. The side chains of the interacting residues are shown in dark gray. The α-carbon trace in the region is shown in light gray. Colipase residues Glu45, Glu64, Arg65, and Asn89 are at the bottom of the figure. Asn366, Gln369, and Lys400 are in the C-terminal domain of PTL. The interacting atoms are connected by dotted lines. The coordinates were obtained from the Protein Data Bank and the figures were prepared with the MOLMOL program (75).

C-terminal domain

The C-terminal has a β-sandwich structure and provides the major binding surface for colipase (43, 44, 64). In the 3-D structures of the PTL-colipase complex, colipase binds to the C-terminal domain. Colipase is a flattened molecule composed of three finger-shaped regions defined by disulfide bridges and belongs to a family of small cysteine-rich proteins that lack extensive secondary structure. In the crystals, the plane of colipase lies almost perpendicular to the center of the C-terminal domain. Two hairpin loops formed by residues 44–46 and 65–87 and Asp89 interact with amino acids in various β-strands of the PTL C-terminal domain (Fig. 5B). Polar interactions, a salt bridge, and hydrogen bonds dominate the interactions.

Colipase interactions with the C-terminal domain

The role of the polar interactions between colipase and the C-terminal domain of PTL has been investigated by site-directed mutagenesis. In the first study, the importance of the ion pair between colipase residue Glu45 and PTL residue Lys400 was tested (65). E45K and E45N colipase and K400E and K400N PTL were created and characterized. Preventing the formation of the ion pair between Glu45 and Lys400 produced PTL-colipase complexes with significantly impaired triglyceride lipase activity. The authors concluded that the ion pair plays a critical role in forming productive PTL-colipase complexes.

The second study looked the effect of introducing mutations in all of the colipase residues that interact with the PTL C-terminal domain (66). A variety of amino acids with different physical properties were substituted for colipase residues Glu45, Glu64, Arg65, and Asp89. These residues form two putative lipase-binding domains, Glu45/Asp89 and Glu64/Arg65. Most of the colipase mutants showed decreased ability to reactivate PTL particularly in the presence of taurodeoxycholate micelles. In particular, mutations in the Glu64/Arg65 binding site caused the greatest effects, but mutations in the other binding site were not well tolerated either. Primarily, the mutations decreased the affinity of the colipase mutants for PTL. One notable exception was E45A, which had normal activity under all conditions. This finding suggests that the ion pair between Glu45 with Lys400 is not essential for complex formation; a conclusion supported by an earlier study that showed the K400A mutant of PTL had normal activity (67). Even so, the results of these studies demonstrate that both the Glu45/Asp89 and Glu64/Arg65 binding sites contribute to complex formation in aqueous solution as predicted by the crystal structure.

Lipid binding region in the C-terminal domain

Recent studies suggest that the C-terminal domain of PTL may have another function apart from binding colipase. Residues 405–414 form a hydrophobic loop, the β5′ loop, that has a large exposed surface area (877 Å^2) of which 488 Å^2 are occupied by hydrophobic side chains (68, 69). Importantly, bound colipase does not mask the hydrophobic surface of the β5′ loop, and the loop is oriented in the same plane as the large hydrophobic plateau comprised by the open lid, the β9 loop, and the tips of the colipase loops. This orientation of the β5′ loop places it in position to participate in lipid binding.

Interestingly, a homologous loop is also present in other proteins that interact with lipids. Lipoprotein lipase, a member of the pancreatic lipase gene family, has a loop in a position analogous to the β5′ loop of PTL, and several studies have demonstrated the importance of the

loop in binding of lipoprotein lipase to lipoproteins and in its activity against triglycerides (70–72). Recently, the protein structures of 15-lipoxygenase and *Clostridium perfringens* α-toxin identified a domain with close structural homology to the β-sandwich structure of the PTL C-terminal domain (69). Additionally, these domains resemble structures of C2 domains, which occur in a number of proteins (69). Both the C-terminal domain of PTL and the C2 domains of other proteins have a β-sandwich structure with three hydrophobic loops (69). Although the β-strand connectivity of the C-terminal domain and the C2 domain of cytosolic phospholipase A2 differ, the β5′ loop of PTL and the third hydrophobic loop on the phospholipase have the same topology. Spectroscopic studies and site-directed mutagenesis have implicated the third hydrophobic loop in binding of the phospholipase A2 to lipid-water interfaces (73, 74). By analogy, the β5′ loop of PTL likely contributes to the interfacial binding properties of the PTL-colipase complex.

Three separate studies provide more direct evidence that the β5′ loop of PTL participates in the interaction of the PTL-colipase complex with an interface. In the first study, monoclonal antibodies that recognized an epitope in the β5′ loop abolished the interfacial activity of PTL. In the presence of the antibodies, PTL hydrolyzed water insoluble substrates poorly, but maintained activity against monomeric substrates. The results were consistent with the hypothesis that the C-terminal domain forms part of the lipid-water interaction site. The other two studies investigated the properties of the isolated C-terminal domain (58, 68). One study showed that the isolated C-terminal domain forms a ternary complex with bile salt micelles and colipase (58). Previous studies by the same group demonstrated that residues in the β5′ loop of PTL interact with the micelle (47, 56). A more recent study indicates that the isolated C-terminal domain, but not the isolated N-terminal domain of PTL, adsorbs to emulsions of trioctanoin and to a phospholipid monolayer in the absence of colipase and bile salts (68). Additionally, the direct involvement of the β5′ loop in interfacial activity was tested by site-directed mutagenesis. For these experiments, a PTL mutant in which the β5′ loop hydrophobicity was increased by substitution of the native sequence with homologous sequence from lipoprotein lipase. The PTL mutant showed more penetration into a monolayer of phospholipids and had increased adsorption at the lipid-water interface in the presence of proteins. These observations support a contribution of the β5′ loop in the interaction of the PTL-colipase complex with an interface.

CONCLUDING REMARKS

The application of techniques from molecular biology and of biophysical methods have greatly advanced our understanding about the molecular details underlying triglyceride hydrolysis by pancreatic lipases. These methods have provided critical insight into the interactions of pancreatic lipase with colipase, bile salts, lipid-water interfaces, and substrates. Still, much remains to be learned about the orientation of the PTL-colipase complex at an interface, the specificity of interactions with bile salts, the details of substrate entrance into the active site, the influence of colipase on lipid organization in the interface, the kinetic differences between PTL and PLRP2 lipases, and the physiological roles of PLRP2 and PLRP1.

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