Val-407 and Ile-408 in the \(\beta5^\prime\)-Loop of Pancreatic Lipase Mediate Lipase-Colipase Interactions in the Presence of Bile Salt Micelles*

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Angela Bourbon Freié, Francine Ferrato, Frédéric Carrière, and Mark E. Lowe

From the Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri 63110, the Laboratoire d’Enzymologie Interfaciale et de Physiologie de la Lipolyse du CNRS, 31 chemin Joseph Aiguier, 13402 Marseille cedex 20, France, and the Departments of Pediatrics and Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine and Children’s Hospital of Pittsburgh, Pittsburgh, Pennsylvania 15213

In a previous study, we demonstrated that the \(\beta5^\prime\)-loop in the C-terminal domain of human pancreatic triglyceride lipase (hPTL) makes a major contribution in the function of hPTL (Chahinian et al. (2002) Biochemistry 41, 13725–13735). In the present study, we characterized the contribution of three residues in the \(\beta5^\prime\)-loop, Val-407, Ile-408, and Leu-412, to the function of hPTL. By substituting charged residues, aspartate or lysine, in these positions, we altered the hydrophilic to lipophilic ratio of the \(\beta5^\prime\)-loop. Each of the mutants was expressed, purified, and characterized for activity and binding with both monolayers and emulsions and for binding to colipase. Experiments with monolayers and with emulsions suggested that the interaction of hPTL with a phospholipid monolayer differs from the interaction of the hPTL-colipase complex with a dicaprin monolayer or a triglyceride emulsion (i.e. neutral lipids). Val-407, Ile-408, and Leu-412 make major contributions to interactions with monolayers, whereas only Val-407 and Ile-408 appear essential for activity on triglyceride emulsions in the presence of bile salt micelles. In solutions of taurodeoxycholate at micellar concentrations, a major effect of the \(\beta5^\prime\)-loop mutations is to change the interaction between hPTL and colipase. These observations support a major contribution of residues in the \(\beta5^\prime\)-loop in the function of hPTL and suggest that a third partner, bile salt micelles or the lipid interface or both, influence the binding of colipase and hPTL through interactions with the \(\beta5^\prime\)-loop.

The three-dimensional structure of human pancreatic triglyceride lipase (hPTL) revealed two functional domains, a globular N-terminal domain and a \(\beta\)-sandwich C-terminal domain (residues 336 – 449) (1). Since the determination of the structure, functional roles have been described for each domain. The N-terminal domain consisting of an \(\alpha/\beta\)-hydrolase fold contains the catalytic site. A surface loop, the lid, delineated by a disulfide bond between Cys-237 and Cys-261, covers the active site and controls the activity of hPTL (2, 3). In the inactive conformation, the lid sterically hinders access of substrate to the active site. In the active conformation, the lid adopts a new position that opens and configures the active site. The C-terminal domain, which has structural similarity to the C2 domain of other lipid-binding proteins, contributes the majority of the binding surface for colipase, a necessary cofactor for hPTL function in the duodenum (4). Until recently, colipase binding was the best documented function for the C-terminal domain.

Over the past several years, several lines of evidence suggested an additional role for the C-terminal domain. Examination of the colipase-PTL crystal structure reveals an exposed hydrophobic loop, the \(\beta5^\prime\)-loop, including residues 405–414, of the C-terminal domain that orients in same plane as the hydrophobic plateau of colipase and the lid (5). This orientation positions the loop to interact with the oil-water surface of the lipid substrate (Fig. 1). Next, a number of studies indicate that a homologous loop in lipoprotein lipase, a protein with structural similarity to hPTL, contributes to the lipid-binding properties of lipoprotein lipase. For instance, mutagenesis of three tryptophans in the lipoprotein lipase loop abolished binding of the lipase to substrate (6). Finally, our earlier study demonstrated that a mutant of hPTL in which the hydrophobicity and size of the \(\beta5^\prime\)-loop was increased by introducing the homologous lipoprotein lipase \(\beta5^\prime\)-loop into hPTL had altered interactions with phospholipid monolayers and with trioctanoin emulsions (7). These findings support a major role for the \(\beta5^\prime\)-loop in the function of hPTL, possibly in the interaction with substrate.

Another finding in our previous study was that the hPTL-\(\beta5^\prime\)-LPL mutant had decreased affinity for colipase in the presence of bile salts (7). Because colipase does not interact with the \(\beta5^\prime\)-loop in the crystal structure of the hPTL-colipase complex, we considered several alternative explanations for the effect of the mutation on the affinity for colipase. One possibility arises from the observation that colipase, PTL, and bile salt micelles can form a complex with the micelle contacting the \(\beta5^\prime\)-loop and residues in colipase (8, 9). \(\beta5^\prime\)-Loop mutations could impair the interaction between PTL and bile salt micelles thereby decreasing the affinity of the hPTL-\(\beta5^\prime\)-LPL mutant for colipase. Another potential explanation is that the larger size of the LPL \(\beta5^\prime\)-loop might sterically hinder binding of the PTL mutant to colipase. Alternatively, the LPL loop could perturb the protein structure near the binding site of colipase resulting in decreased affinity for colipase even though the overall structure of the hPTL-\(\beta5^\prime\)-LPL mutant is not grossly altered.

In this study, we have addressed the possibility that the \(\beta5^\prime\)-loop interacts with a third partner to increase the affinity for colipase by introducing mutations in the \(\beta5^\prime\)-loop that increase the hydrophobic/ lipophilic balance of the loop without greatly altering the size. To accomplish these studies, we created mutants of hPTL with substitutions of a positively or negatively charged residue for a hydrophobic residue. Each mutant was expressed in yeast, purified, and characterized for interfacial binding and lipase activity in both bulk phase.

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1 To whom correspondence should be addressed: Dept. of Gastroenterology, Children’s Hospital of Pittsburgh, 3705 Fifth Ave., Pittsburgh, PA 15213. Tel: 412-692-9412; Fax 412-692-8906; E-mail: mark.lowe@chp.edu.

2 The abbreviations used are: hPTL, human pancreatic triglyceride lipase; hPTL5153G, hPTL mutant in which the active site serine 153 has been substituted with a glycine residue; TDC, taurodeoxycholate; LPL, lipoprotein lipase.
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and monolayer assays. The effects of bile salts and colipase on activity and binding were studied and compared with the properties of hPTL.

EXPERIMENTAL PROCEDURES

Site-directed Mutagenesis—Manipulations of DNA were done by using standard methods (10). Mutations were introduced into the wild-type hPTL cDNA in the pHILS1 vector by PCR using the Stratagene QuikChange site-directed mutagenesis kit per the manufacturer’s protocol (11). Oligonucleotide primers were designed to introduce the desired mutations and were generally 27 bp long. Transformations into Epicurian Coli XL-1 Supercompetent cells were performed as described in the QuikChange protocol. Minipreps were performed using either the Qiagen Plasmid Mini kit or the Qiagen QIAprep Miniprep kit. Presence of the desired mutation was confirmed by dideoxynucleotide sequence analysis using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit. The DNA was transformed into yeast by electroporation, and positive clones were selected as previously done (11, 12).

Protein Methods—The mutant lipases were expressed and purified as previously described (11). Protein concentrations were determined by amino acid analysis and by spectrophotometry at 280 nm using an extinction coefficient of 1.2. The purified proteins were analyzed by SDS-PAGE and staining with GELCODE Blue stain (Pierce) to confirm the homogeneity of the isolated protein.

Lipase Activity Measurements—The lipase activity of wild-type and mutant hPTL was determined in bulk by measuring the release of fatty acids from mechanically stirred emulsions of tributyrin, trioctanoin, or triolein as previously described (7, 13). Unless otherwise stated, pure colipase from porcine pancreas or pure recombinant, human colipase was added at a molar excess of 5. The lipolytic activities are expressed in International Units per milligram of enzyme. One unit corresponds to 1 μmol of fatty acid released per minute.

Binding of hPTL and the Mutants to Tributyrin and Trioctanoin Emulsions—The interfacial binding of hPTL and the mutants was assayed by mixing the enzyme with a trioctanoin or tributyrin emulsion as previously described (14, 15). A 5-fold molar excess of colipase and varying amounts of taurodeoxycholate were included in the incubations as indicated.

Monolayer Experiments—Lipase activities were measured by the monolayer technique using monomolecular films of 1,2-dicaprin (Fluka, París, France) spread over an aqueous phase made of 10 mM Tris-HCl buffer, pH 8.0, 100 mM NaCl, 21 mM CaCl₂; 1 mM EDTA, and placed in a zero order trough (16). The hydrolysis rates were expressed in moles of substrate hydrolyzed per cm² of the reaction compartment, per minute, and for a theoretical enzyme concentration of 1 μM in the reaction compartment.

The kinetics of the adsorption of hPTL and β5'-loop mutants were studied by recording the changes with time of the surface pressure of a monomolecular film of egg phosphatidylcholine (PC, Sigma, Saint Quentin-Fallavier, France) spread at the air-water interface in a cylindrical Teflon trough (volume: 5 ml; surface area: 7 cm²). The surface pressure was measured using the Wilhelmy method with a thin platinum plate (perimeter 3.94 cm) attached to an electromicrobalance (Beckman LM 600). The trough was filled with 10 mM Tris-HCl buffer (pH 8.0), 100 mM NaCl, 21 mM CaCl₂; 1 mM EDTA, and the monolayer was manipulated using various volumes (1–10 μl) of a 1 mg ml⁻¹ phospholipid solution in chloroform. Once the initial surface pressure had stabilized, the lipase was injected into the aqueous subphase, which was stirred for 30 s at 250 rpm with a magnetic rod to homogenize the aqueous phase. The increase in the surface pressure was then recorded for 60 min. Experiments with PC monolayers were performed using 5 μg of lipase (20 μM overall concentration in the trough).

Modeling of β5'-Loop Mutations and Calculating the Water-accessible Surfaces—Three-dimensional models of the hPTL-β5'-loop mutants were built from the known x-ray three-dimensional structure of open hPTL (accession number 1LPA in the Protein Data Bank). The water-accessible surfaces were calculated using the DSSP option in the Turbo-Frodo software program (17, 18).

RESULTS

Design and Expression of Single Substitution Mutants in the β5'-Loop—Examination of the three-dimensional structure of hPTL revealed that three hydrophobic residues, Val-407, Ile-408, and Leu-412, reside on the surface of the β5'-loop (Fig. 1). To determine the effect of increasing the hydrophilic to lipophilic ratio of the β5'-loop without greatly changing the size of the loop, we substituted each site

TABLE 1
Water-accessible surfaces (Å²) of the β5'-loop in hPTL and the β5'-loop mutants

|             | HPTL | V407D | I408D | L412D | V407K | I408K | L412K |
|-------------|------|-------|-------|-------|-------|-------|-------|
| Charged residues | 105  | 277   | 289   | 286   | 283   | 290   | 286   |
| Semipolar residues | 284  | 206   | 264   | 185   | 246   | 311   | 237   |
| Hydrophobic residues | 488  | 409   | 315   | 384   | 409   | 320   | 384   |
| Total surfaces    | 877  | 892   | 865   | 855   | 938   | 921   | 907   |
| HLB*             | 0.80 | 1.18  | 1.75  | 1.23  | 1.29  | 1.88  | 1.36  |

* HLB is the hydrophilic/lipophilic balance and is defined by the (charged plus semipolar/hydrophobic) ratio.
with an aspartic acid to create I407D, V408D, and L412D or with lysine to create I407K, V408K, and L412K. Introduction of a single charged residue changed the accessible surface area minimally, 2.5% or less for the aspartate mutants and 7% or less for the lysine mutants (Table 1). In contrast, the hydrophilic to lipophilic ratio was increased from 50% to 135%, with substitutions at position 408 causing the greatest effect.

Each mutant was expressed in *Pichia pastoris* and purified as described under “Experimental Procedures.” The yeast secreted each mutant protein into the medium indicating that the proteins were not misfolded. The yield for each mutant ranged from 5 to 10 mg/liter of culture. Analysis of the purified proteins by SDS-PAGE revealed a single band for each mutant (data not shown). By this analysis, there was no evidence for degradation of any of the mutants. We further confirmed the structural integrity of each mutant by determining the temperature stability of the mutants. After incubation at 50 °C for 20 min, similar residual activity, ~50% of the original activity in 0.5 mM TDC, remained for wild-type hPTL and each of the hPTL mutants. Additionally, the activity and binding of the mutants did not change over time indicating that degradation or alterations such as isoaspartate formation in the substituted residues did not occur (19).

**Effect of Colipase and Bile Salts on the Activity of the βS’-Loop Mutants against Three Different Substrates**—To begin our analysis of the mutants, we measured the activity of hPTL and each mutant against emulsions of tributyrin, trioctanoin, and triolein in the standard pH-stat assay containing increasing concentrations of TDC. In the absence of...
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**TABLE 2**

| Lipase   | Activity (units/mg) | Activity (units/mg) |
|----------|---------------------|---------------------|
| Wild type| 100%                | 100%                |
| V407D    | 95%                 | 95%                 |
| I408D    | 88%                 | 88%                 |
| L412D    | 80%                 | 80%                 |
| V407K    | 75%                 | 75%                 |
| I408K    | 60%                 | 60%                 |
| L412K    | 40%                 | 40%                 |

**TABLE 3**

| Lipase              | Tributyrin: Activity (units/mg) | Tributyrin: Activity (units/mg) |
|---------------------|---------------------------------|---------------------------------|
| Wild type           | 50%                             | 50%                             |
| V407D               | 45%                             | 45%                             |
| I408D               | 40%                             | 40%                             |
| L412D               | 30%                             | 30%                             |
| V407K               | 25%                             | 25%                             |
| I408K               | 20%                             | 20%                             |
| L412K               | 10%                             | 10%                             |

**Partitioning of the βS'-Loop Mutants in Tributyrin and Trioctanoin**

We next tested the ability of the βS'-loop mutants to partition with lipid substrate at both 0.5 and 4.0 mM TDC in the presence of colipase (Table 3). At 0.5 mM TDC, the mutants partitioned with tributyrin and trioctanoin to the same extent as did wild-type hPTL. In the presence of 4.0 mM TDC, clear differences among the mutants were seen. V407D, I408D, and V407K had decreased partitioning with both tributyrin and trioctanoin, a finding consistent with the decreased or absent activity of these mutants against these lipids. I408K partitioned well in tributyrin and has ~60% of wild-type activity (Fig. 2). I408K also partitioned normally in trioctanoin even though the mutant had no activity against trioctanoin in 4.0 mM TDC and colipase (Fig. 2). The L412D and L412K mutants had normal partitioning in both lipids as expected for their near normal activity against tributyrin and trioctanoin. These results support a role for the βS'-loop in the function of hPTL in the presence of micellar solutions of bile salts.

**Interaction of the βS'-Loop Mutants with Colipase**

One explanation for the decreased activity and partitioning of the mutants in 4.0 mM TDC is that the βS'-loop mutations impair the interactions of the mutant lipases with colipase. To test the effects of the mutations on the colipase-lipase interaction, we first determined the ability of colipase to activate the βS'-loop mutants over a range of colipase concentrations. We were able to calculate the apparent dissociation constant \( K_d \) and apparent binding constant \( B_{max} \) for the colipase-hPTL complex in 4.0 mM TDC by nonlinear regression with a rectangular hyperbola function (Table 4). The apparent \( B_{max} \) was similar to hPTL for all of the mutants. The two Leu-412 mutants had values for apparent \( K_d \) similar to hPTL. In contrast, the I408K mutant had a 5-fold increase in \( K_d \) and the V407K, V407D, and I408D mutants had 5-fold increases in \( K_d \) values. These results indicate that mutations in positions 407 and 408 influence the interaction of colipase and hPTL in the presence of bile salt micelles but not in the presence of bile salt monomers.

We next tested the ability of each mutant to compete for colipase with hPTL or with S153G, an inactive mutant of hPTL with normal colipase binding. In each of these assays, we included equimolar amounts of the βS'-loop mutants with a lipase:colipase ratio of 1:0.5. Because the V407D, I408D, and V407K mutants have no activity against trioctanoin in 4 mM TDC, we tested their ability to compete with hPTL in an assay against trioctanoin in 4 mM TDC. None of these mutants inhibited hPTL under these conditions indicating that they have a much lower affinity for colipase than does hPTL (Table 5). For the mutants with activity at 4.0 mM TDC (I408K, L412D, and L412K), we estimated their affinities for colipase by their ability to compete with S153G lipase for colipase. If these mutants have normal or near normal affinities for colipase, S153G should decrease their activity by 50%. In this assay, S153G decreased L412D activity to 47.0 ± 8.2%, I408K activity to 46.2 ± 17.1%, and L412K activity to 31.3 ± 21.4%. Although there was considerable variability in the assay for the I408K and L412K mutants, it is clear these mutants can...
compete with S153G for colipase and have relatively preserved affinity for colipase. The two methods concur that the V407D, I408D, and V407K mutants have decreased affinity for colipase in 4.0 mM TDC, whereas the L412D, I408K, and L412K mutants have normal or near normal affinities for colipase.

**Effect of the β5’-Loop Mutations on Lipase Activity against Monolayers**—To more directly determine the interaction of the mutants with a lipid interface, we turned to studies with monolayers. Monolayers allow the variation and control of the interfacial quality of the interface and allow assays in the absence of colipase and bile salts (16). We first measured the activity of the mutants against monolayers of dicaprin. Each mutant was assayed at various surface pressures ranging from 15 to 35 millinewtons/m (Fig. 4). In general, the mutants behaved similarly to hPTL. Although the V407D, V407K, and L412D mutants had increased activity compared with hPTL, the differences were not statistically significant. These results indicate that the introduction of single mutations into the β5’-loop did not greatly alter the

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**TABLE 4**

| Lipase | Apparent $K_d$ (μM) | Apparent $B_{max}$ (μmol/min/mg) |
|--------|----------------------|----------------------------------|
| Wild type | 4.7 ± 0.8 | 11,800 ± 640 |
| V407D | 72.0 ± 6.0 | 10,740 ± 450 |
| I408D | 74.0 ± 4.6 | 10,855 ± 245 |
| L412D | 6.9 ± 0.9 | 9,890 ± 360 |
| V407K | 84.9 ± 5.6 | 11,380 ± 400 |
| I408K | 14.0 ± 1.1 | 10,790 ± 310 |
| L412K | 6.8 ± 1.2 | 10,270 ± 565 |

**TABLE 5**

| Lipase | Residual lipase activity (%) |
|--------|------------------------------|
| **A) Incubation of the mutant lipase with hPTL and colipase** | |
| V407D | 107.6 ± 7.8 |
| I408D | 116.0 ± 16.7 |
| V407K | 117.8 ± 27.6 |
| **B) Incubation of the mutant lipase with colipase followed by the addition of S153G hPTL** | |
| L412D | 47.0 ± 8.2 |
| I408K | 46.2 ± 17.1 |
| L412K | 38.9 ± 18.5 |

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**FIGURE 3.** The colipase dependence of the β5’-loop mutants. The assays were done by the pH-stat method. Each assay included 1.3 nm lipase, 310 mM tributyrin, and the indicated concentrations of colipase and TDC. Activity is micromoles of fatty acid released per min per mg lipase. The symbols are defined in the inset.
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FIGURE 4. Activity of the β5’-loop mutants against a dicaprin monolayer. The activities were determined on a KSV-2200 barostat using a zero order Teflon trough as described under “Experimental Procedures.” Each lipase was injected to a final concentration of 0.04–0.065 nM. The change in surface pressure was determined at various starting pressures for each lipase. Each point is the mean ± S.D. of three or more determinations. The symbols are defined in the inset.

FIGURE 5. Penetration/adsorption of the β5’-loop mutants onto an egg phosphatidylcholine monolayer. 17 μg of lipase was injected under the monolayer, and the increase in surface pressure was measured. The maximum increase in surface pressure (∆π) was plotted as a function of the initial surface pressure (π). Both ∆π and π are expressed in millinewtons per meter. The linear curves were obtained by linear regression using the least squares method. The symbols are defined in the inset.

interaction of the mutant lipases with a neutral lipid monolayer and suggest that the β5’-loop plays a greater role in the activity against neutral lipids in the presence of colipase and bile salts.

We next measured the interaction of the β5’-loop mutants with a zwitterionic monolayer of phosphatidylcholine. For our analysis of interactions with phosphatidylcholine, we determined the increase in surface pressure as a function of the initial surface pressure of the monolayer under which the lipase was injected (Fig. 5). Phosphatidylcholine is not a substrate for hPTL or the mutants, and the recorded variations in surface pressure only reflect the ability of the test lipase to bind and penetrate the phospholipid monolayer. Each of the β5’-loop mutants behaved similarly. They all showed decreased penetration compared with hPTL. None of the mutants penetrated the monolayer above a surface pressure of 4 millinewtons/m, whereas the critical surface pressure of penetration for hPTL was above 12 millinewtons/m.

DISCUSSION

In our previous study, we concluded that the β5’-loop plays a critical role in the function of hPTL (7). In the present study, we provide evidence that the β5’-loop, particularly the region containing Val-407 and Ile-408, contributes to the function of hPTL by influencing the binding of colipase in micellar concentrations of bile salts. Additionally, experiments with monolayers and with emulsions suggest that the interaction of hPTL with a phospholipid monolayer differs from the interaction of the hPTL-colipase complex with a dicaprin monolayer or a triglyceride emulsion (i.e. neutral lipids).

Importance of Residues Val-407 and Ile-408—The substitution mutants of Val-407 and Ile-408 had greater effects than the replacement of Leu-412. Both the aspartate and lysine substitutions at position 407 greatly impaired the activity of the mutant PTL in the presence of bile salt micelles. In contrast, the aspartate substitution at position 408 made a greater change in activity than did the lysine substitution. Furthermore, the I408K mutant had relatively preserved partitioning with tributyrin and trioctanoin as well as only minimal effects on the interaction with colipase. The differences between I408D and I408K lipases can be explained by repulsion of the negatively charged aspartic side chain from the negatively charged surface of an emulsion particle coated with TDC or of TDC micelles. Conversely, the positively charged side chain of lysine might interact favorably with these same surfaces. Alternatively, the hydrophobic portion of the lysine side chain might stretch or snorkle into the hydrophobic portion of the emulsion or bile salt micelle and thereby allow the I408K mutant to have near normal function (20). Regardless, the data are consistent with the conclusion that the region of the β5’-loop containing Val-407 and Ile-408 contributes to the function of hPTL.

Binding to Phospholipid Monolayers—A major difference between the results of experiments with a phospholipid monolayer and with triglyceride emulsions is the importance of Leu-412 in the interactions with phospholipid monolayers and the relative lack of effect with emulsions and dicaprin monolayers. Like the other mutants, the Leu-412 mutants showed decreased penetration of a phosphatidylcholine monolayer compared with hPTL. In contrast, Leu-412 does not appear to contribute significantly to the activity of hPTL in the presence of triglyceride emulsions with bile salts with or without colipase. Leu-412 mutations had minimal to no effect on the activity against tributyrin, trioctanoin, and triolein, on binding to tributyrin and trioctanoin, or on the interactions with colipase.

The varied behavior of the Leu-412 mutants depending on the nature of the lipid-water interface suggests that hPTL may orient differently on a phospholipid monolayer than it does on an emulsion particle. Molecular modeling of the hPTL indicates that Leu-412 should not be as close
to the interface as are Val-407 and Ile-408 when the βS'-loop and the putative lipid binding regions of the N-terminal domain (lid and β9-loop) are in contact with the interface. If hPTL positions so that Leu-412 interacts with the interface, the catalytic site moves away from the interface creating an unfavorable orientation of hPTL for activity. Improper orientation of hPTL when bound to a phospholipid interface could explain, in part, the absence of activity of hPTL against phospholipids and the ability of phospholipids to inhibit hPTL activity in either monolayers or emulsions.

The βS'-Loop and Colipase—In our earlier study, where we replaced the βS'-loop of hPTL with the homologous loop from LPL, we also found that the mutant hPTL had reduced affinity for colipase (7). We offered several possible explanations for these findings. First, the larger lipoprotein lipase βS'-loop might sterically hinder the binding between the mutant hPTL and colipase. Alternatively, the presence of the larger βS'-loop could cause enough perturbation of the C-terminal domain structure to alter the critical colipase binding sites in this domain. The current study makes these interpretations less likely. The substitutions do not greatly alter the size of the βS'-loop, and the single-site substitutions are less likely to perturb the colipase binding sites of the C-terminal domain. Examination of the crystal structure of the hPTL-colipase complex indicates that the substitution of either asparagine or lysine at Val-407 or Ile-408 should not allow interactions of the new side chains with colipase. More importantly, each of these mutants showed normal activity and normal partitioning with colipase in 0.5 mM TDC. These results suggest that the βS'-loop mutants hPTL can form normal interaction with colipase under these conditions. Thus, the single-site substitutions we created and characterized do not hinder the interaction between colipase and hPTL because of their size or by perturbing the structure of the C-terminal domain.

Another explanation for the decreased affinity of the LPL βS'-loop mutant for colipase arose because we only measured the interaction between colipase and the mutant by competition with S153G hPTL. Under these conditions, the S153G-colipase complex may have a stronger affinity for the interface than does the lipoprotein lipase βS'-loop hPTL-colipase complex. Even if the mutant hPTL has normal affinity for colipase in the bulk phase, the accumulation of the S153G-colipase complex indicates that the substitution of either aspartate or lysine at Val-407 or Ile-408 should not allow interactions of the new side chains with colipase. More importantly, each of these mutants showed normal activity and normal partitioning with colipase in 0.5 mM TDC. These results suggest that the βS'-loop mutants hPTL can form normal interaction with colipase under these conditions. Thus, the single-site substitutions we created and characterized do not hinder the interaction between colipase and hPTL because of their size or by perturbing the structure of the C-terminal domain.

The last hypothesis to explain our results is that colipase and hPTL are mixed micelles. In contrast to pure bile salt micelles, fatty acid-bile salt micelles increase the binding between colipase and hPTL 1000-fold (29). In fact, several studies have shown the formation of a hPTL-colipase complex with mixed micelles or with the lipoprotein particles present in bile (22, 30–32). In our experimental system, fatty acid-bile salt micelles may form rapidly during a short and lag period or the bile salt micelle-hPTL-colipase complex may form at the lipid-water interface. In vivo, the hPTL-colipase complex likely forms on the mixed micelles present in bile (22). Pancreatic and biliary secretions mix before they enter the duodenum. Because pancreatic secretions contain colipase and hPTL at concentrations of 10^{-6} M and the dissociation constant for the hPTL-colipase complex in the presence of mixed micelles is 10-fold lower, binding to the mixed micelles in bile is likely to be rapid.

Although there is evidence that colipase and hPTL are mixed micelles, there is no evidence that colipase and hPTL remain associated with the colipase-hPTL complex and allows optimal activity. Whatever the nature of the complex at the lipid-water interface, the present results confirm that the colipase-hPTL association is stabilized in a ternary complex influenced by residues Val-407 and Ile-408 (Fig. 1).

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