Colipase Residues Glu\textsuperscript{64} and Arg\textsuperscript{65} Are Essential for Normal Lipase-mediated Fat Digestion in the Presence of Bile Salt Micelles*  

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Wallace V. Crandall§ and Mark E. Lowe¶  
From the Departments of Pediatrics and of Molecular Biology and Pharmacology, Washington University School of Medicine and St. Louis Children’s Hospital, St. Louis, Missouri 63110

Pancreatic triglyceride lipase (PTL) requires colipase for activity. Various constituents in meals and in bile, particularly bile acids, inhibit PTL. Colipase restores activity to lipase in the presence of inhibitory substances like bile acids. Presumably, colipase functions by anchoring and orienting PTL at the oil-water interface. The x-ray structure of the colipase-PTL complex supports this model. In the x-ray structure, colipase has a hydrophobic surface positioned to bind substrate and a hydrophilic surface, lying opposite the hydrophobic surface, with two putative lipase-binding domains, Glu\textsuperscript{45}/Asp\textsuperscript{89} and Glu\textsuperscript{64}/Arg\textsuperscript{65}. To determine whether the hydrophilic surface interacts with PTL in solution, we introduced mutations into the putative PTL binding domains of human colipase. Each mutant was expressed, purified, and assessed for activity against various substrates. Most of the mutants showed impaired ability to reactivate PTL, with mutations in the Glu\textsuperscript{64}/Arg\textsuperscript{65} binding site causing the greatest effect. Analysis indicated that the mutations decreased the affinity of the colipase mutants for PTL and prevented the formation of PTL/colipase complexes. The impaired function of the mutants was most apparent when assayed in micellar bile salt solutions. Most mutants stimulated PTL activity normally in monomeric bile salt solutions. We also tested the mutants for their ability to bind substrate and anchor lipase to tributyrin. Even though the ability of the mutants to anchor PTL to an interface decreased in proportion to their activity, each mutant colipase bound to tributyrin to the same extent as wild type colipase. These results demonstrate that the hydrophilic surface of colipase interacts with PTL in solution to form active colipase-PTL complexes, that bile salt micelles influence that binding, and that the proper interaction of colipase with PTL requires the Glu\textsuperscript{64}/Arg\textsuperscript{65} binding site.

Lipases participate in a variety of physiologic and industrial processes (1, 2). They aid in the preparation of foods and pharmaceuticals. They serve to generate second messengers, participate in cytokotoxicity reactions, and have become a model system for the study of molecular interactions occurring at lipid-water interfaces. Most importantly, they allow for the digestion and trafficking of dietary triglycerides and phospholipids by converting apolar dietary lipids to more polar products, fatty acids and monoacylglycerols. An understanding of the digestive process has implications for the treatment of human diseases, including malnutrition and obesity.

The digestion of dietary triglyceride begins in the stomach through the process of emulsification and the actions of gastric lipase (3). Digestion continues in the duodenum where pancreatic triglyceride lipase (PTL) releases 50–70% of dietary fatty acids (3). Although PTL requires bile acids to function efficiently, bile acid micelles inhibit PTL unless another pancreatic protein, colipase, is present (2). Colipase restores PTL activity in the presence of physiologic concentrations of bile acids. Without colipase, PTL could not cleave fatty acids from dietary triglycerides, and fat malabsorption with its consequences would result.

Previous models of colipase function uniformly proposed that colipase acts as a bridge between PTL and the lipid substrate by binding to both PTL and the lipid emulsion surface. The strongest support for this model comes from the crystal structures of the porcine colipase-human PTL complex solved under different conditions (4, 5). In the first structure, PTL resides in an inactive conformation with the catalytic site sterically blocked by a surface loop, called the lid. The other structure, solved in the presence of mixed micelles, reveals the active conformation of PTL in which the lid moves away from the catalytic site and forms new interactions with colipase. Colipase has similar conformations in both structures (6). Importantly, colipase has two opposing surfaces that can potentially anchor PTL to the substrate. One surface, which contains predominantly hydrophilic residues, forms interactions with the C-terminal domain of PTL. The opposite surface, which faces away from PTL, has four hydrophobic loops positioned to interact with the substrate surface. In this conformation, colipase brings PTL into the proper proximity and orientation for lipid hydrolysis.

The crystal structure predicts that van der Waals forces and several polar bonds stabilize the interaction between colipase and PTL. The number of putative polar bonds varies between the inactive and active conformation of PTL. In the active conformation of PTL, two colipase residues, Glu\textsuperscript{55} and Arg\textsuperscript{58}, form polar bonds with residues in the PTL lid. In both conformations of PTL, polar interactions occur between five residues of colipase, Arg\textsuperscript{44}, Glu\textsuperscript{55}, Glu\textsuperscript{64}, Arg\textsuperscript{65}, and Asn\textsuperscript{69}, and main-chain residues in the C-terminal domain of PTL. Four residues form two potential binding domains, one containing residues 45

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§ Present address: Division of Pediatric Gastroenterology and Nutrition, Columbus Children’s Hospital, 700 Children’s Dr., Columbus, OH 43205.

¶ To whom correspondence should be addressed: Washington University School of Medicine, 660 South Euclid Ave., Campus Box 8208, St. Louis, MO. Tel.: 314-286-2857; Fax: 314-286-2894; E-mail: Lowe@kids.wustl.edu.

1 The abbreviations used are: PTL, pancreatic triglyceride lipase; CMC, critical micelle concentration; TBST, Tris-buffered saline with Tween 20; TDC, taurodeoxycholate; BSA, bovine serum albumin; PAGE, polyacrylamide gel electrophoresis.
and 89, which interact with Lys\textsuperscript{400} of PTL, and the other containing residues 64 and 65, which interact with Gln\textsuperscript{369} of PTL. Arg\textsuperscript{44} is not conserved and is replaced by a serine or methionine in most other species. Additionally, Arg\textsuperscript{44} does not form a polar bond in the porcine colipase-PTL complex. These points argue that Arg\textsuperscript{44} may not play an important role in colipase-PTL interactions in solution.

A few studies have examined the role of specific amino acids in the formation of the colipase-PTL complex. One study examined the interaction between Glu\textsuperscript{15} of colipase and the PTL lig by site-specific mutagenesis of Glu\textsuperscript{15} (7). This study concluded that the interactions between Glu\textsuperscript{15} and the PTL lig stabilized the active confirmation of PTL. Two other studies investigated the interaction between colipase and the C-terminal domain of PTL. Jennens and Lowe (8) introduced substitution and deletion mutations into the C-terminal domain of PTL. Single substitution mutations of multiple PTL residues thought to interact with colipase did not affect lipase activity. Deletion of the C-terminal domain greatly decreased PTL activity, but the truncated PTL still required colipase for activity in the presence of bile acids. These mutants raised questions about the importance of the interactions between colipase and the C-terminal domain of PTL. Another recent study provided support for the predicted interaction between one colipase residue, Glu\textsuperscript{65}, and Lys\textsuperscript{400} of the PTL C-terminal domain (9). Ayvazian et al. (9) demonstrated that disruption of this interaction impaired lipase activity secondary to an inability of the mutant colipase to bind PTL. None of these studies addressed the relative importance of the other putative lipase binding residues in the hydrophilic surface of colipase.

To determine if the hydrophilic surface of colipase interacts with lipase in solution and to identify participating residues, we introduced mutations into positions 44, 45, 64, 65, and 89 of human colipase by site-specific mutagenesis. Each mutant colipase was characterized for the ability to reassociate PTL in the presence of bile salts and to anchor PTL at an oil-water interface. The results support the orientation of colipase as defined by the crystal structures. That is, the hydrophilic surface of colipase interacts with PTL in solution. Furthermore, the results identify the 64/65 binding domain, specifically position 64, as key to the normal function of colipase, thereby adding additional details to the mechanism of PTL-mediated lipolysis.

MATERIALS AND METHODS

Construction of Mutants—Manipulations of DNA were done by standard methods (10). Mutations were introduced into wild type human colipase cDNA in the pPIC9 vector by polymerase chain reaction using the Stratagene QuikChange site-directed mutagenesis kit per the manufacturer’s protocol. Oligonucleotide primers were designed to introduce the desired mutations. Transformations into E. coli XL-1 Blue Supercompetent cells were performed per the QuikChange protocol. Minipreps were prepared using either the Qiagen Plasmid Mini kit or the Qiagen QuickPrep 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.

Protein Methods—Proteins were expressed and purified as previously described (11). Protein concentrations were determined by ultraviolet spectrophotometry at A\textsubscript{280} using an extinction coefficient of 0.47. Equal quantities (3 \textmu g) of each mutant protein were run on SDS-PAGE gels and stained with GELCODE Blue stain (Pierce, Rockford, IL) to confirm the homogeneity of the protein product.

Stability in urea and TDC was determined for colipase and the colipase mutants. Each colipase was incubated for 30 min at room temperature in 8 M urea or 4 mM TDC in 50 mM Tris-HCl, pH 8.0, at a concentration of 0.1 mg/ml. Companion incubations were done in buffer alone. After the incubation, 500 ng of the colipase was assayed with 2.8 \textmu g of PTL using tributyrin emulsified with 0.5 mM TDC as described below. The difference between PTL activity in the absence and presence of colipase was defined as the colipase-stimulated PTL activity. Neither colipase nor the colipase mutants lost any function after incubation in buffer for 30 min at room temperature.

Colipase Assays—Assays were performed at 37 °C using a VIT 90 Radiometer pH-stat, in assay buffer containing 1 mM Tris-HCl, pH 8, 2 mM CaCl\textsubscript{2}, and 4 mM TDC. 2800 ng of wild type lipase and 560 ng of mutant lipase was added to 1 ml of binding buffer in a 1.5-ml microcentrifuge tube that had been precoated with 1 mg/ml BSA in binding buffer. The samples were immediately placed on ice and stored at \(-20^\circ\text{C}\). The amount of lipase remaining in the aqueous phase was determined by activity assay using 200 \textmu l of the aqueous phase and 270 ng of wild type lipase and 560 ng of mutant colipase were added to 1 ml of binding buffer in a 1.5-ml microcentrifuge tube that had been precoated with 1 mg/ml BSA in binding buffer. The samples were vortexed for 1 min, then centrifuged at 14,000 rpm for 5 min. 800 \mu l of the upper, aqueous phase was carefully removed and placed in a microcentrifuge tube that had also been precoated with 1 mg/ml BSA in binding buffer. The samples were immediately placed on ice and stored at \(-20^\circ\text{C}\). The amount of lipase remaining in the aqueous phase was determined by activity assay using 200 \mu l of the aqueous phase and 270 ng of wild type lipase. The amount of lipase bound to the substrate surface was calculated as the difference between the amount of lipase initially added and the amount recovered in the aqueous phase. We developed a radioimmunoposay to determine the amount of colipase remaining in the aqueous phase (see below). The amount of procolipase bound to the substrate was calculated as the difference between the total amount initially added, and the amount remaining in solution.

Radioimmunoposay for Colipase Quantitation—The amount of procolipase bound to the interface was determined by radioimmunoposay. 100-\mu l aliquots of the aqueous phase were tested for each mutant. The
RESULTS

Colipase Mutants—The crystal structures of the pig colipase-human PTL complex identified polar interactions between residues Arg^{44}, Glu^{45}, Glu^{64}, Arg^{65}, and Asn^{80} of porcine colipase and residues in the C-terminal domain of PTL (6). Although the interactions clearly form in crystals, the same bonds may not stabilize the colipase-PTL complex in solution. To determine whether or not these interactions contribute to the binding between human colipase and PTL in solution, we created a variety of single- and double-substitution mutants in human colipase (Fig. 1). Initially, we substituted a small neutral amino acid, alanine, in positions Glu^{45}, Glu^{64}, and Arg^{65}, which are conserved in human colipase, and in positions Ser^{44} and Asp^{80}, which are not conserved. We next substituted amino acids with identical charge but different side-chain length, with opposite charge in the side chain or with a bulky side chain to determine which properties of the side chain influence binding of colipase to PTL. Each of the mutant proteins was expressed in a yeast system and purified by immunopurification and gel filtration chromatography. Analysis of the purified proteins by SDS-PAGE revealed a single broad band for each protein indicative of an uncontaminated purified protein suitable for further characterization (Fig. 2).

Activity of Colipase Mutants at Various TDC Concentrations—The effect of bile salts on PTL varies depending on the concentration of the bile salts (2, 14). Below their critical micelle concentration (CMC) bile salts stimulate PTL activity. Above their CMC bile salts inhibit PTL activity. Colipase increases PTL activity in both cases, indicating that colipase and PTL interact below and above the CMC of a given bile salt. Although colipase and PTL probably form a complex at all bile salt concentrations, the interactions stabilizing the complex in the presence of bile salt monomers could differ from those in the presence of bile salt micelles. Thus, we tested the ability of the colipase mutants to stimulate PTL activity at various concentrations of TDC, which has a CMC of about 1.1 mM under the assay conditions (Fig. 3). In the absence of colipase, the activity of PTL increased at 0.5 mM TDC and, then, dropped sharply as the TDC concentration increased (Fig. 3, A, ●; B, ●). The addition of native colipase increased PTL activity at all TDC concentrations (Fig. 3, ●). The S44A mutant restored full PTL activity at all TDC concentrations tested, suggesting that Ser^{44} does not contribute to the binding of human colipase to human PTL (Fig. 3A, ○). The other alanine mutants separated into two groups by putative binding site (Fig. 3A). Below the CMC, single alanine substitutions into the Glu^{45}/Asp^{80} and Glu^{64}/Arg^{65} binding sites had little effect on PTL activity compared with native colipase. Both double-substitution mutants had decreased function but still increased PTL activity above the no colipase levels. By 4 mM TDC, the E64A and R56A mutants restored less than 5% of the PTL activity compared with native colipase, and the E64A/R56A double mutant restored no activity to PTL. The function of the single-substitution mutants in the other binding site remained relatively preserved even at 4 mM TDC. The E54A restored full activity to PTL, and the D89A restored 45% of activity. Mutating both Glu^{45} and Asp^{80} decreased the function of the double mutant to about 5% of native colipase. These findings indicate that both sites on colipase contribute to the binding between colipase and PTL on colipase. Of the two sites, Glu^{64}/Arg^{65} appears to form essential interactions with PTL.

We extended these observations with studies on additional...
colipase mutants. These mutants were created to alter the physical properties of the side chains in specific ways. We changed the side-chain charge, length, and bulk in Glu45, Asp89, Glu64, and Arg65. Several of these mutants, E45D, E45Q, and D89R, did not express well and could not be analyzed. Of the remaining mutants, only the substitution of lysine for arginine at position 65 was tolerated. PTL had full activity with the R65K colipase mutant (Fig. 3B, E). The other mutants had relatively preserved function below the CMC of TDC compared with their greatly decreased function above the CMC. At position 45, the substitution of a bulky side group abolished the ability to restore PTL activity above the CMC of TDC (Fig. 3B, f). Likewise, the substitution of a negatively charged amino acid, glutamic acid, for a positively charged amino acid, arginine, at position 65 eliminated function above the CMC (Fig. 3B, m). Position 64 was sensitive to both chain length and charge with both the E64D and E64Q mutant colipases showing decreased function (Fig. 3B, v and ˅). The results confirm the importance of the targeted sites in the interaction between colipase and PTL.

**Stability of the Mutants**—Decreased stability of the mutants in 4 mM TDC could also explain the above results. To test the stability of the mutants, we incubated them in 8 M urea or 4 mM TDC for 30 min at room temperature. After the incubation we determined the ability of colipase and each colipase mutant to activate PTL. Under both conditions native colipase retained full function, as did each of the colipase mutants. These results make it unlikely that any of the mutations altered the stability of colipase to a significant degree.

**Concentration Dependence of the Alanine Mutants**—The assays at various TDC concentrations suggested that bile salt micelles might affect formation of productive complexes between colipase and PTL. To better characterize any differences in the interaction between colipase and PTL in the presence and absence of bile salt micelles, we measured the ability of the colipase mutants to reactivate PTL over a range of colipase concentrations in the presence of excess substrate and a constant amount of PTL (Fig. 4). From these data, we determined the concentration of the colipase mutants that restored half-maximal activity to PTL ($K_d$) and the concentration that restored maximal PTL activity ($B_{max}$) in 0.5 and 4 mM TDC. The apparent $K_d$ and $B_{max}$ were determined by nonlinear regres-
The assays contained 0.5 or 4.0 mM TDC and equimolar measure of the relative function among the colipase mutants. Parameters cannot be easily measured. Still, parameters require of a cofactor (14). As a result, true Michaelis-Menten substrate concentrations (Fig. 5). Enzymatic lipolysis does not also affect the interaction of the colipase-PTL complex to sub-
titions indicated that the mutations did not grossly disrupt the structure of the colipase mutants.

At 4 mM TDC, we see differences in the curves (Fig. 4, C-F). The curves shift to the right for most of the mutants indicating a change in affinity of the colipase mutants for PTL. The apparent Bmax for all mutants equals that of native colipase, whereas the apparent Kd increased for all of the mutants (Table II). Mutations of the Glu64/Arg65 binding site affected the apparent Kd to a greater degree than mutations of the Glu64/Asp69 binding site. The greatest effect was seen with the R65E mutant, which had an apparent Kd value ~4-fold weaker than wild type (Table I). The preserved function of the colipase mutations under these conditions indicated that the mutations did not grossly disrupt the structure of the colipase mutants.

Substrate Dependence of the Colipase Mutants—To assure that mutations of the hydrophilic surface of colipase did not also affect the interaction of the colipase-PTL complex to sub-
strate, we measured the activity of the mutants over a range of substrate concentrations (Fig. 5). Enzymatic lipolysis does not lend itself to standard steady-state analysis due to the inter-
facial mechanism, constantly changing substrate, and the re-
requirement of a cofactor (14). As a result, true Michaelis-Menten parameters cannot be easily measured. Still, parameters determined under constant, predetermined conditions provide a measure of the relative function among the colipase mutants.

The assays contained 0.5 or 4.0 mM TDC and equimolar amounts of PTL and the colipase mutants. In 0.5 mM TDC, wild type colipase and all of the colipase mutants increased PTL activity to the same level. In 4.0 mM TDC, two mutants, E45A and R65K, had curves identical to that of native colipase (Fig. 5, A, B). With the remaining mutants, PTL had decreased maximal velocities. These impressions were confirmed by calculation of the apparent Km and maximal Vmax of the colipase-PTL complex for tributyrin (Table III). The mutants and wild type colipase had similar values for Km and Vmax in 0.5 mM TDC. The complex produced with all tested mutants in 4.0 mM TDC had an apparent Km equal to that of the complex with wild type colipase. E45A and R65K-PTL complexes had normal apparent maximal velocities. Complexes with the remaining mutants had decreased apparent Vmax. PTL had low activity with E64A, and the data did not provide a good fit. Consequently, the apparent constants were not reported, but the shape of the curve suggested that the E64A mutation primarily affected the maximal velocity. The decreased apparent Vmax indicated that some of the mutants form fewer productive complexes with PTL in 4.0 mM TDC than does native colipase, a finding consistent with decreased affinity of the colipase mutants for PTL. Once a complex forms between the various mutant colipases and PTLs, the complex has a normal affinity for the substrate. That is, the mutations in the hydro-
philic surface of colipase do not affect the binding of the complex to the substrate interface once the complex has formed.

**TABLE I**

| Mutant | Bmax (μmol/min) | Kd (nM) |
|--------|-----------------|---------|
| WT     | 4671 ± 440      | 1.03 ± 0.27 |
| E45A   | 4667 ± 380      | 0.66 ± 0.17 |
| D89A   | 4474 ± 217      | 0.63 ± 0.10 |
| E64A   | 4202 ± 68       | 0.97 ± 0.05 |
| E64D   | 4660 ± 531      | 1.19 ± 0.25 |
| E64Q   | 4148 ± 265      | 1.19 ± 0.25 |
| R65A   | 4210 ± 126      | 0.94 ± 0.10 |
| R65K   | 4204 ± 428      | 0.66 ± 0.22 |
| R65E   | 3613 ± 166      | 4.37 ± 0.42 |

**TABLE II**

| Mutant | Bmax (μmol/min) | Kd (nM) |
|--------|-----------------|---------|
| WT     | 7635 ± 533      | 0.8 ± 0.2 |
| E45A   | 8560 ± 334      | 1.3 ± 0.2 |
| D89A   | 8269 ± 155      | 6.5 ± 0.4 |
| E64A   | 9036 ± 830      | 45.8 ± 12.7 |
| E64D   | 7981 ± 192      | 9.5 ± 0.85 |
| E64Q   | 7404 ± 330      | 30.8 ± 4.7 |
| R65A   | 8471 ± 392      | 22.7 ± 3.8 |
| R65K   | 7095 ± 131      | 1.7 ± 0.1 |
| R65E   | 7111 ± 237      | 256.8 ± 24.9 |

**DISCUSSION**

In the crystal structure of porcine colipase bound to PTL, polar interactions form between colipase residues Glu64, Asn69 (Asp69 in human colipase), Glu65, and Arg66 and residues in the C-terminal domain of PTL (6). We mutated each putative lipase-binding residue of colipase to determine whether these amino acids participate in complex formation when the proteins are in solution. Characterization of the colipase mutants showed that substitutions in each targeted site decreased the ability of colipase to bind PTL in the presence of bile salt.
micelles. Several conclusions can be made from these results. First, residues in the hydrophilic surface of colipase mediate the formation of colipase-PTL complexes in solution as predicted by the crystal structure. Second, bile salt micelles influence the assembly of productive colipase-PTL complexes. Third, both the Glu45/Asp89 and Glu64/Arg65 binding domains contribute to complex formation, but the Glu64/Arg65 binding site predominates. Although the Glu45/Asp89 sites tolerated alanine substitutions, any changes, with the exception of a conserved mutation at position 65, in the Glu64/Arg65 binding site greatly diminished the activity of these colipase mutants when assayed above the CMC of TDC.

The increased sensitivity of the Glu64/Arg65 site compared with the Glu45/Asp89 site was not expected, based on the crystal structure of the PTL-colipase complex (6). The carboxylate side chain of Glu64 forms a hydrogen bond with the main-chain nitrogen of PTL residue Gln369 and serves as a contact point for a water molecule bridge involving Glu45 of colipase and Ser366 of PTL. Arg65 contributes another hydrogen bond with the side chain of Gln369. In contrast, Glu45 forms the only salt bridge with the C-terminal domain of PTL (Lys400), forms a hydrogen bond with Asn366, and contributes to the water bridge noted above. Asp89 has a hydrogen bond with Lys400 of PTL and contributes to a water bridge with Gly66 of colipase and Leu444 of PTL. Additionally, site-specific mutagenesis of Glu45 and Lys400 of PTL suggested that the ion pairing between these two residues was essential for lipolysis (9). Our results question this conclusion. The preserved function of the E45A colipase mutant suggests that the salt bridge between Glu45 of colipase and Lys400 of PTL is not essential or, alternatively, may not form in solution.

Regardless of which binding site was mutated, bile salts altered the behavior of most single substitution mutants. The magnitude of the effect depended on the bile salt concentration.

| Mutant | $V_{\text{max}}$ (0.5 mM TDC) | $K_m$ (0.5 mM TDC) | $V_{\text{max}}$ (4.0 mM TDC) | $K_m$ (4.0 mM TDC) |
|--------|-----------------------------|-------------------|-----------------------------|-------------------|
| WT     | 14800 ± 890                | 2.4 ± 0.5         | 13572 ± 292                | 1.6 ± 0.1         |
| E45A   | 15660 ± 1560               | 2.7 ± 0.9         | 12999 ± 491                | 2.0 ± 0.3         |
| D89A   | 15500 ± 1180               | 1.7 ± 0.5         | 7386 ± 502                 | 1.5 ± 0.4         |
| E44A   | 14600 ± 1250               | 1.5 ± 0.6         | ND                         | ND                |
| E46D   | 15600 ± 1520               | 1.7 ± 0.7         | 4269 ± 138                 | 1.6 ± 0.2         |
| E46Q   | 14900 ± 1100               | 1.5 ± 0.5         | 1779 ± 51                  | 1.1 ± 0.1         |
| R65A   | 15500 ± 1450               | 3.1 ± 0.9         | 1783 ± 100                 | 1.2 ± 0.3         |
| R65E   | 13800 ± 1200               | 1.5 ± 0.5         | ND                         | ND                |
| R65K   | 15950 ± 900                | 1.9 ± 0.4         | 13042 ± 330                | 1.9 ± 0.2         |

$^a$ ND, not determined.
At 0.5 mM TDC, when monomers of bile salts spread at the oil-water interface, the affinity of the colipase mutants for PTL was unchanged except for R65E. At higher TDC concentrations, above the CMC, the affinity of the colipase mutants decreased appreciably for all but the E45A and S44A mutants. At least two models can be offered to explain this observation. The first model proposes that colipase and PTL must form a complex with bile salt micelles in aqueous solution before binding to the interface. There is support for this model in the literature. Multiple studies have demonstrated that bile salt micelles or multiple bile salts bind to colipase and colipase-PTL complexes of PTL and bile salt micelles (20–22). In another study, monomeric bile salt solutions allowed the absorption of PTL to an interface, but a micellar bile salt solution completely inhibited PTL binding to an interface (23). Two kinetic studies suggested that bile salt micelles or multiple bile salts bind to PTL and inhibit absorption of PTL to the substrate-water interface even in the presence of colipase (24, 25).

Both models could account for our data. Mutations in the hydrophilic surface of colipase could destabilize the complex of PTL, bile salts and colipase predicted to form by in the first model. This disruption could occur because the colipase mutants have decreased affinity for the PTL-bile salt complex or because the mutants bind to PTL in an incorrect conformation producing an inactive complex. The fact that the colipase mutants have lowered affinity for PTL and can bind to the substrate but not anchor PTL even as an inactive complex argues against the last possibility. The weakened binding affinity of the colipase mutants for PTL is also consistent with the second model. In this instance the decreased affinity of the colipase mutants for PTL allows the bile salt micelles to compete for PTL binding, and thereby inhibit the formation of an active colipase-PTL complex.

Although the role of bile salt micelles in the lipolytic mechanism remains unclear, many molecular details of lipolysis have been described in the past decade. X-ray crystallography and site-specific mutagenesis have broadened the role of colipase. Monolayer experiments, kinetic analysis, and site-specific mutagenesis identified the catalytic triad of lipases with mutations in the lid have demonstrated a role for the lid in substrate selectivity, in substrate binding, and interactions with colipase. Monolayer experiments, kinetic analysis, and site-specific mutagenesis have broadened the role of colipase in the lipolytic mechanism. It stabilizes the lid in the open, active conformation and may direct PTL to substrate patches on the surface of dietary emulsions, which contain high concentrations of phospholipids and cholesterol esters. This work extends these observations to better define the crucial interactions between colipase and PTL. An increased understanding of the molecular details of this important physiologic process provides the information needed to rationally modulate PTL activity for therapeutic purposes.

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