C-terminal domain of human pancreatic lipase is required for stability and maximal activity but not colipase reactivation

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Abstract Fungal lipases and human pancreatic lipase (hPL) share a common tertiary structure termed the α/β hydrolase fold. In contrast, the region C-terminal to the common tertiary structure does not share any common structural features with fungal lipases, leading to the hypothesis that the divergent C-terminal domain confers specific properties to hPL. To study the role of the C-terminal domain in hPL function, we made substitution and deletion mutations in the C-terminal domain. The mutant proteins were expressed in transfected COS-1 cells and the secreted proteins were analyzed by immunoblot and for lipase activity. Substitution mutants in multiple lysine residues, in aspartate 390, or in tyrosine 404 did not affect secretion or lipase activity of the mutants. Significantly, the mutants still required colipase for maximal activity. Deletion of the C-terminal domain decreased the amount of truncated, mutant protein in the medium of transfected cells and decreased the specific activity of the mutants. Still, maximal activity required colipase, indicating that the deletion mutants interacted with colipase. Interfacial binding of the truncated deletion mutants was decreased relative to wild-type hPL. The newly synthesized deletion mutants were not as efficiently secreted from the transfected cells as wild-type hPL, and the mutant proteins that appeared in the medium were less stable than the wild-type hPL. These findings suggest that the C-terminal domain is required for proper folding or processing of hPL, confers stability, and increases activity, but is not absolutely required for colipase reactivation of the bile salt-inhibited enzyme.—**Jennens, M. L., and M. E. Lowe.** C-terminal domain of human pancreatic lipase is required for stability and maximal activity but not colipase reactivation. *J. Lipid Res.* 1995. **36:** 1029–1036.

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Lipases, which cleave triglycerides into free fatty acids and acylglycerols at oil–water interfaces, have been isolated from numerous sources. These enzymes have a variety of sizes, substrate specificities, stereo specificities, and catalytic rates. Comparisons of the primary structure of lipases from fungi with human pancreatic lipase (hPL) revealed few similarities other than the Gly-X-Ser-X-Gly motif that includes the active site serine (1). This amino acid sequence is found in proteases and esterases as well as other lipases that contain the nucleophile–histidine–acid catalytic triad (2). The lack of amino acid sequence homology suggested that fungal and mammalian lipases belong to different structural classes.

This concept changed dramatically after the three-dimensional structures of human pancreatic lipase (hPL) and several fungal lipases were solved (3–5). These lipases shared a similar topology and three-dimensional structure in the central β-sheet and the topology of the catalytic triad residues was conserved. Comparisons to other enzymes showed that the fold pattern, the α/β hydrolase fold, was conserved among a number of hydrolases (1). The similarities lead to the suggestion that these enzymes evolved from a common ancestral hydrolase (1).

The region C-terminal to the conserved α/β hydrolase fold is quite divergent in structure and may confer specific properties to each of the individual enzymes. The fungal lipases have loops of varying lengths in the C-terminus that form a single domain with the central β-sheet. In contrast, hPL contains a separate C-terminal domain consisting of a β-sandwich structure (3). This domain is encoded by the last three exons of the gene suggesting that it was added during evolution (6). The gene organization and the protein structure of the C-terminal domain suggest that this region may confer a unique function to hPL.

We investigated the role of the C-terminal domain in hPL function by creating mutations in the domain. Single or double amino acid mutations and deletion mutations were made and the mutant proteins were expressed in mammalian cells. The mutant lipases were tested for colipase dependent activity, stability, and secretion rates.

Abbreviations: h, human; PL, pancreatic triglyceride lipase; PLRP, pancreatic lipase-related protein; PCR, polymerase chain reaction.

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METHODS

Construction of mutants and expression in COS-1 cells

All manipulations of DNA were by standard methods (7). Mutations were introduced into the cDNA for hPL by the PCR overlap method (8, 9). Synthetic oligonucleotides were obtained from the Oligonucleotide and Protein Chemistry Laboratory at Washington University School of Medicine. The fidelity of each mutant and of the PCR product was determined by the dideoxy chain termination method. The numbering system for hPL is used throughout this manuscript.

The mutant cDNAs were subcloned into pSVL and transfected into COS-1 cells by the DEAE dextran method (9). Twenty four hours after transfection the medium was removed, replaced with serum-free medium, and harvested 72 h post-transfection for analysis. The medium was analyzed directly or, for some experiments, concentrated 10- to 20-fold with an Amicon macrosolute concentrator.

Analysis of expressed proteins

The presence of expressed protein in the medium was assessed by SDS-PAGE, transfer to PVDF membrane, and detection with a polyclonal antibody against hPL as described previously (9). Lipase assays were done in triplicate with [^{3}H]triolein emulsified with taurodeoxycholate and extracted as before (9). Interfacial binding to tributyrin was determined as previously described (9). Recombinant human procolipase or colipase was prepared by a published method and added to the assay as indicated (10). hPL amounts were quantitated by immunoblot as previously described (9).

Pulse-chase

Secretion rates were determined in cells that were 72–96 h post-transfection. The serum-free culture medium was aspirated and the cell layer was washed 3 times with serum-free, methionine-free medium. They were then incubated in serum-free, methionine-free medium containing 250 μC/ml [^{35}S]methionine for 20 min at 37°C. The medium was replaced with medium containing a 10× concentration of cold methionine. Medium and cell pellets were collected at indicated times and the expressed protein was purified by immunoprecipitation as described. The immunoprecipitates were analyzed by SDS-PAGE and fluorography as described previously (11). The amount of protein at each time point was quantitated by laser densitometry.

RESULTS

Lysines and colipase activation

A number of studies have suggested that colipase binds to the C-terminal domain of PL (12–16). In particular, various methods, including chemical modification and X-ray crystallography have identified specific lysine residues that may mediate PL binding to colipase. To determine whether a lysine residue plays a crucial role in the binding of PL to colipase, we systematically mutated each lysine residue that was conserved in human, pig, and rat PL including the C-terminal domain lysines implicated in colipase binding.

Substitution mutants were constructed at each target lysine and each mutant protein was expressed in COS-1 cells. The medium from the transfected cells was analyzed by SDS-PAGE followed by immunoblot and by lipase activity measurements. The mutant lipases were secreted from the transfected cells as determined by immunoblot analysis (data not shown). Lipase activity was detected in the medium of cells transfected with each mutant (**Table 1**). Importantly, taurodeoxycholate inhibited each mutant and each mutant was reactivated by colipase. There were no significant differences in the specific activity among the mutants, indicating that the mutations did not affect activity or the interaction with colipase. Thus, no single lysine residue was critical for colipase reactivation. In particular, Lys400, the residue that formed a salt bridge with Glu45 of procolipase in the crystal structure of the procolipase-hPL complex, was not required for colipase reactivation (16).

Asp390 and Tyr404 mutations

The crystal structure of the pig procolipase-hPL complex identified two other C-terminal domain residues in addition to Lys400 that interacted with colipase (16). Tyr404 provided an apolar interaction by stacking with Arg65 of procolipase and Asp390 formed a salt bridge with Ser44 of the pig procolipase. Examination of the human procolipase sequence revealed that Ser44 is not con-

TABLE 1. Lipase activity of the hPL lysine mutants

Lipase	No Colipase	Colipase
	μmol fatty acid released/min/μg protein	
Wild-type	ND	7.3 ± 0.8
K70A	ND	6.9 ± 0.6
K81A	ND	6.7 ± 0.8
K92A	ND	7.1 ± 0.5
K108A	ND	6.4 ± 1.0
K240A	ND	7.5 ± 0.4
K269A	ND	6.2 ± 0.5
K296A	ND	7.7 ± 0.9
K318A	ND	7.3 ± 0.6
K351A	ND	6.8 ± 0.7
K400A	ND	6.9 ± 0.5

The activity of the expressed lipases was determined against triolein as described in Methods. About 100 ng of each lipase was assayed with a 2-fold molar excess of recombinant colipase. The values are the average of three determinations each done in triplicate ± the SD; ND, none detected.

TABLE 2. Lipase activity of the Asp390 and Tyr404 hPL mutants

Lipase	No Colipase	Colipase
	μmol fatty acid released/min/μg protein	
Wild-type	ND	8.4 ± 0.7
D390A	ND	7.9 ± 0.8
Y404A	ND	8.2 ± 0.5

The activity against triolein was determined with about 100 ng of each lipase in the assay. A 2-fold molar excess of colipase was added as indicated. The results are the average of four determinations ± SD; ND, none detected.

TABLE 3. Lipase activity of the double mutants

Lipase	No Colipase	Colipase
	μmol fatty acid released/min/μg protein	
Wild-type	ND	7.7 ± 0.9
D390A/K400A	ND	7.2 ± 0.7
K296A/K400A	ND	7.7 ± 0.6

Activity was determined as described in the Methods. About 100 ng of each lipase was assayed with a 2-fold molar excess of recombinant colipase. The values are the average of three determinations ± SD; ND, none detected.

served and is an arginine. Modelling of Arg44 suggests that it or other nearby residues could form salt bridges with Asp390.

To determine whether either Asp390 or Tyr404 is crucial for hPL binding to colipase, both were mutated to alanines. The mutant cDNAs were expressed in COS-1 cells and the medium was analyzed for secretion of the mutant hPL. Each mutant was detected in the medium by immunoblot (data not shown) and lipase activity (**Table 2**). Taurodeoxycholate inhibited the activity of both mutants and colipase restored activity in both mutants to specific activities that were indistinguishable from the wild-type. This analysis demonstrated that neither Asp390 nor Tyr404 is critical for hPL to successfully interact with colipase.

Double mutants of hPL

We next tested two double mutants to determine whether mutations in more than one site were necessary to inhibit the interaction with colipase. One mutant, Asp390Ala/Lys400Ala, was chosen because these two residues interact with colipase in the crystal structure (16, 17). The other mutant, Lys296Ala/Lys400Ala, was chosen because the loop containing Lys296 is near colipase in the open-lid conformation of the crystal structure. Both mutants were secreted into the medium of transfected cells and activity could be easily detected (**Table 3**). Each mutant was inhibited by bile salt and activity was restored by the addition of colipase to the assay, indicating that each double mutant interacts with colipase.

C-terminal deletion mutants

Because of the difficulty in identifying specific amino acids that are involved in hPL interaction with colipase, another approach was taken. We concentrated on the C-terminal domain that was implicated in colipase binding by other studies, and created deletions in that domain by introducing stop codons at various positions to produce truncated hPL mutants. One stop codon was placed after Phe432, deleting the disulfide bridge at the extreme C-terminal end of the protein and a short region of amino acids that is poorly conserved among members of the lipase family. Another stop codon was placed after Asp388, deleting a region of great divergence among lipase family members and implicated in colipase binding in horse PL. Finally, two mutants were made to remove the entire C-terminal domain. One was truncated at the chymotrypsin cleavage site, Phe336, because chymotrypsin digestion releases the intact C-terminal domain, and the other was truncated at an exon–intron junction, Tyr341 (6, 13).

The activity of each mutant was determined after expressing the proteins in transfected COS-1 cells. The two hPL mutants truncated within the C-terminal domain, Phe432 and Asp388, were not secreted by the transfected cells although the recombinant proteins were detected in the cell pellets (**Fig. 1**). No lipase activity was detected in the medium or in extracts of the cell pellets (**Table 4**). These findings suggested that an intact C-terminal domain is critical for proper folding or processing of hPL.

In contrast, the truncated mutants with the entire C-terminal domain were secreted into the medium as faster

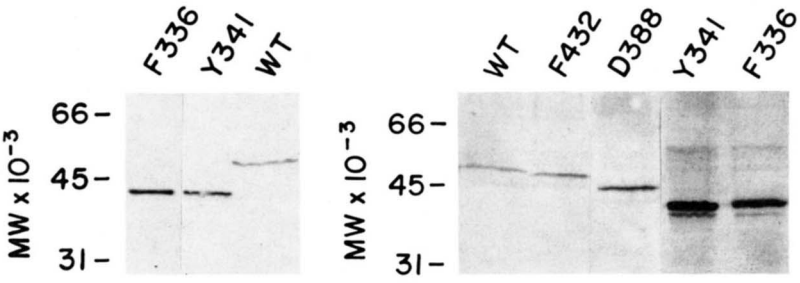

Fig. 1. Expression of hPL and the Phe336 and Tyr341 truncated lipases in COS-1 cells. The proteins were expressed in COS-1 cells. The medium and cells were analyzed by immunoblot. Left panel: the medium for the truncated mutants was concentrated 20-fold in comparison to the medium containing the wild-type (WT) lipase; F336, hPL truncated after Phe336; Y341, hPL truncated after Tyr341. Right panel: the cell pellets for wild-type hPL and each truncated mutant; F432, hPL truncated after Phe432; D388, hPL truncated after Asp388.

TABLE 4. Lipase activity of the truncated lipases

Lipase	No Colipase	Colipase
	μmol fatty acid released/min/μg protein	
Wild-type	ND	7.8 ± 0.3
F336[a]	ND	1.3 ± 1.5
Y341[a]	ND	1.8 ± 1.6
D388[a] cells	ND	ND
D388[a] medium	ND	ND
F432[a] cells	ND	ND
F432[a] medium	ND	ND

Lipase was determined with triolein as the substrate. About 50 ng of each lipase with a 2-fold molar excess of colipase was included in the assay. The values are the average of twenty separate determinations ± SD; ND, none detected.

[a]Indicates a stop codon is present after the residue.

migrating proteins on SDS-PAGE, reflecting their smaller size (Fig. 1). The amounts found in the medium were generally 10-fold less than for the wild-type, but the mutants were readily detectable. The presence of the mutants in the medium suggested that the secreted proteins were properly folded and processed by the COS-1 cells.

Both the Phe336 and the Tyr341 mutants had lipase activity in the presence of colipase. The specific activity of the mutants was 4-fold less than the wild-type on average, but the range of values fluctuated greatly (Table 4). The activity was inhibited by taurodeoxycholate or deoxycholate and restored by colipase. The decreased specific activity of the deletion mutants indicated that the C-terminal domain contributes to the activity of the protein either through effects on the conformation of the N-terminal domain or by directly participating in the reaction. These findings also showed that the truncated mutants formed functional interactions with colipase despite missing the entire C-terminal domain and suggested the N-terminal domain interacted effectively with colipase.

Interfacial binding and colipase interaction of Tyr341

During the course of these studies, we noted that single time point assays gave differing specific activities depending on the length of the incubation time. Longer incubation times gave higher specific activities than shorter incubation times. An explanation for this observation was provided by measuring the time course of the reaction for Tyr341 (**Fig. 2A**). When no colipase was included in the assay, no activity was present. When colipase was added to the incubation, the rate of triolein hydrolysis increased after a lag time (Fig. 2A). There are several explanations for the lag time observed with the Tyr341 mutant. The deletion mutant may not bind to the interface efficiently, the conformational changes required to form the oxyanion hole may not occur easily, or the mutant may not interact with colipase properly.

To determine whether there are important differences in the interaction between the Tyr341 mutant and colipase, we measured the dependence of the Tyr341 mutant's activity on colipase concentration. The activity depended on the molar ratio of colipase to the mutant, increasing linearly until about a 1:1 ratio when the rise in activity slowed considerably (Fig. 2B). The curve for the mutant

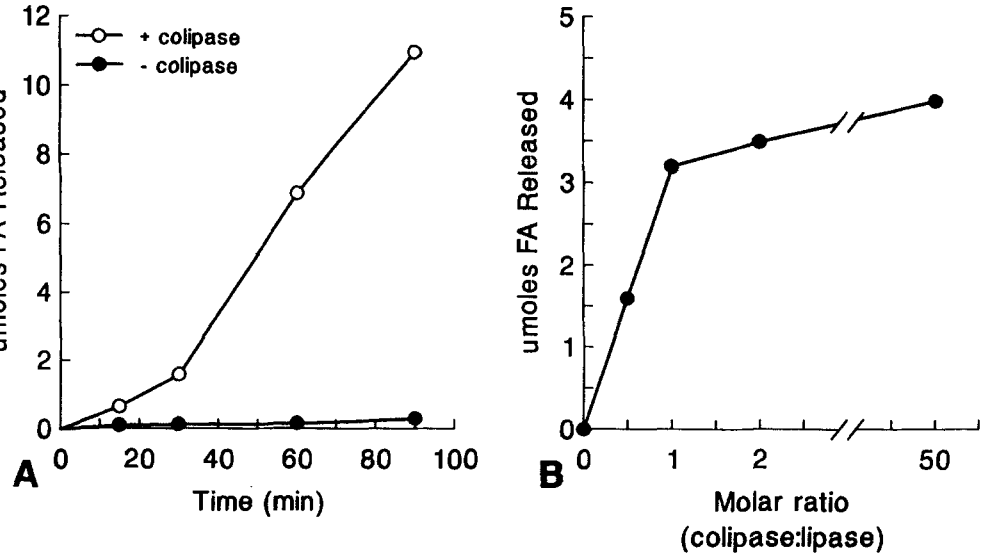

Fig. 2. The colipase dependence of the Tyr341 truncated mutant hPL and hPL. Activity was determined with triolein as the substrate. Panel A shows the release of fatty acids as a function of time. Fifty ng of the mutant lipase was included in each assay point; closed circles, hPL with colipase; triangles, Tyr341 with 2-fold molar excess of colipase; squares, Tyr341 without colipase. Each point is the average of two experiments. Panel B shows the dependence of lipase activity for Tyr341 and hPL at various ratios of colipase to the mutant. Fifty ng (2.5×10^{-8} M) of the mutant hPL and hPL was present in the assay; squares, hPL; circles, Ty341 deletion mutant. Each point is the average of two experiments.

was similar to the curve for wild-type hPL. This finding suggested that the interaction of the Tyr341 mutant with colipase was not markedly weaker than that of intact hPL with colipase and was consistent with the possibility that colipase reactivation of hPL involves interactions with residues in the N-terminal domain.

We next determined the ability of the Tyr341 mutant to bind to interfaces formed by tributyrin, using a centrifugation assay that separated mixtures of tributyrin and buffer into organic and aqueous phases. When tributyrin alone was included in the binding buffer, wild-type hPL was almost completely associated with the organic phase (**Table 5**). Tyr341 bound to a much lesser extent over the same time period. More of the mutant was bound with a longer incubation, but the amount bound was still well below the amount of wild-type hPL bound. The addition of taurodeoxycholate to the incubation inhibited binding of wild-type hPL and the addition of colipase to the incubation restored binding. Colipase improved the binding of Tyr341 to the interface. Still, the amount bound was 4-fold less than for wild-type hPL at 5 min and 3-fold less at 10 min. These findings suggest that interfacial binding of the truncated mutants is decreased and that the decreased interfacial binding, even with colipase present, might account for the lag phase seen in activity measurements.

TABLE 5. Wild-type hPL and Y341* binding to tributyrin

Additions	Wild-Type (2 min)	Y341[a] (5 min)	Y341[a] (10 min)
	% remaining in aqueous phase		
None	100	100	100
TB	2	95	69
TB + TDC	73	94	88
TB + TDC + colipase	15	68	46

Binding was done as previously described (9). Each determination is the average of two determinations done in duplicate. The duplicates varied by 10% or less for each point. The time given is the length of incubation with a tributyrin emulsion prior to centrifugation; TB, tributyrin; TDC, taurodeoxycholate.

[a]Indicates a stop codon is present after the residue.

Secretion of the truncated mutants

The observation that the amount of truncated lipases present in the medium of transfected COS-1 cells was 10-fold lower than the amount found for wild-type hPL can be explained in several ways. The mutants may not fold as efficiently as the wild type hPL and most of the synthesized protein remains in the cells. The amount synthesized may be less or the secreted protein may be more sensitive to degradation in the medium.

To determine the secretion rate of the truncated Tyr341 mutant and wild-type hPL from COS-1 cells, the secretion of each protein from cells and their subsequent appearance into the medium was determined by pulse-labeling followed by chase of newly synthesized protein. The 0 time values for the mutant and the wild-type were quite similar, 0.75 ± 0.05 and 0.69 ± 0.07 absorbance units, respectively. Secretion of the mutant was only slightly slower than secretion of wild-type hPL during the first hour (**Fig. 3A**). During the last hour of the experiment, very little of the mutant was secreted from the cells

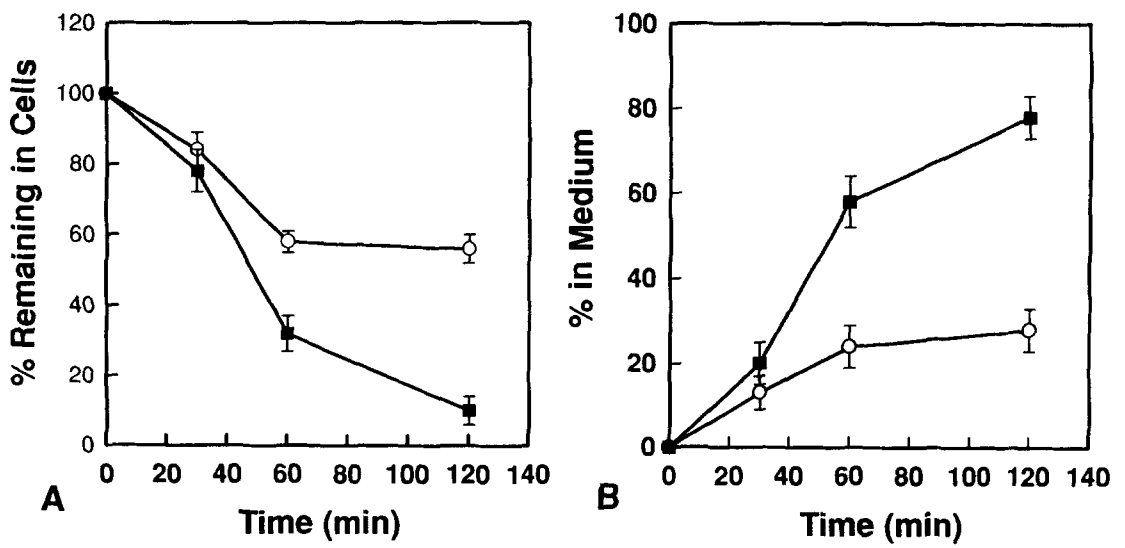

Fig. 3. The secretion of the Tyr341 truncated hPL mutant from transfected COS-1 cells. COS-1 cells were transfected with Tyr341 truncated mutant and the wild-type hPL and radiolabeled with [^{35}S]methionine as described in Methods. Samples were analyzed by immunoprecipitation, SDS-PAGE, and fluorography at the indicated times. The relative amounts were determined by laser densitometry and the values are expressed as a percentage of the amount in the cells at time zero. Panel A: intracellular expressed proteins. Panel B: extracellular expressed proteins; open circles, Tyr341 truncated hPL; closed squares, wild-type hPL.

and about 50% remained with the cell in contrast to the wild-type where only 10% remained in the cell at 2 h. The appearance of the mutant and wild-type hPL in the medium showed the same pattern (Fig. 3B). These data suggested that 50% of the newly synthesized hPL truncation mutant was secreted at a rate comparable to the wild-type hPL and must be efficiently folded and processed for secretion. The remainder of the deletion mutant was not properly folded or processed for secretion. Thus, one determinant of decreased secretion was folding or processing of the mutant, processes affected by the C-terminal domain.

Stability of the truncated mutants

To determine whether the secreted, truncated mutants were more labile than wild-type hPL, we incubated medium containing Tyr341 or wild-type hPL at 4°C and assayed aliquots at intervals. Activity of the Tyr341 truncated hPL decayed with a half-life of about 5 h and there was only 5% of the original activity remaining at 12 h (**Fig. 4**). By comparison, the wild-type hPL showed no loss of activity over the 12-h time period and showed only a 10% loss of activity when tested after 2 wk at 4°C. The Tyr341 mutant had greatly decreased stability. Immunoblot analysis of the Tyr341 mutant remaining in solution demonstrates that there was a loss of mass, suggesting that the mutant was more susceptible to degradation by endogenous proteases than was wild-type hPL. The rapid loss of activity in the Tyr341-truncated mutant could account for the wide range of specific activities that we observed for these mutants and suggested that the C-terminal domain is necessary to maintain a functional conformation of the protein. Together, decreased secretion of the deletion mutant and the decreased stability account for the decreased amount of intact mutant in the medium.

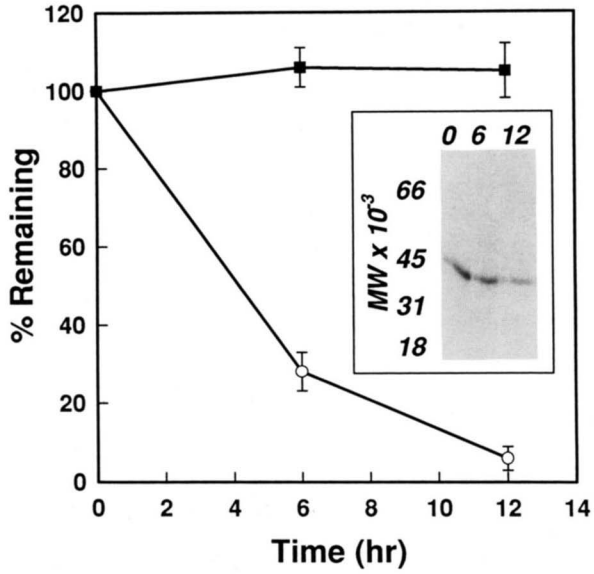

Fig. 4. Decay of Tyr341 activity with time. Concentrated medium containing identical amounts of the Tyr341 truncated hPL and wild-type hPL as judged by immunoblot were incubated at 4°C and aliquots were assayed for activity at 0, 6, and 12 h; open circles, Tyr341 truncated hPL; closed squares, wild-type hPL. The insert shows an immunoblot of the Tyr341 truncated hPL remaining in the medium at each time point.

DISCUSSION

In this study, we investigated the role of the divergent C-terminal domain of hPL in the function of hPL. Deletion mutants of the C-terminal domain suggested that this region of hPL was not required for a functional interaction with colipase, but the C-terminal domain was critical for maximal activity and stability of hPL. Deleting only a portion of the C-terminal domain prevented secretion of the mutants from transfected cells, suggesting that the truncated mutants did not fold properly. Deleting the entire domain allowed secretion of the N-terminal domain in an active, but unstable, form. Although there were no direct interactions of the C-terminal domain with the N-terminal domain in the crystal structure of hPL alone or in complex with procolipase, our data indicate that an intact C-terminal domain is necessary to stabilize and maintain the structure of the N-terminal domain (3, 16).

No single lysine residue, including the lysine that formed a salt bridge with colipase in the crystal structure, out of 10 lysines examined in this study was essential for colipase reactivation of hPL nor did this study confirm a role for Tyr404 and Asp390, residues that interacted with procolipase in the crystals of the procolipase–hPL complex (16). Mutations in any of these residues or double mutants of Lys296/Lys400 and of Asp390/Lys400 did not affect the functional interaction of the mutant hPL with colipase as determined by the ability to reactivate bile salt inhibited hPL. If colipase does form a salt bridge with a lysine residue and make contacts with Tyr404 and Asp390, those interactions are not essential for function.

There are several explanations to reconcile this finding with the multiple studies that implicated various lysine residues in PL binding to colipase and the X-ray crystallography study that implicated Tyr404 and Asp390 in colipase binding (16). First, any salt bridges or crosslinking of PL lysines with colipase residues may be fortuitous and not indicative of a stable interaction because the long side chain on lysine provides a high degree of flexibility and movement permitting interactions with multiple residues on colipase. Second, the binding of PL with colipase in solution may be mediated by more than one residue and the residues that participate in the interaction may depend on the conditions of the assay. This plasticity may allow other residues to substitute for the mutated amino acid. Such interactions may actually be enhanced in the lysine mutants where the smaller alanine side chain may allow in-

teractions with colipase that are normally constrained when the bulkier lysine residue is present. Third, the interaction of colipase with PL may be different in solution than the interaction that forms at an oil–water interface. Binding studies of colipase and PL support this hypothesis by showing that the binding of colipase to PL was stronger in the presence of an interface than in solution (18, 19). Finally, there may be differences in the interactions of colipase and PL from various species that account for the differences in binding residues reported in the literature. For instance, the binding affinity of pig colipase for pig PL was weaker than the affinity of human colipase for hPL (20). Clearly, the binding of colipase to PL involves more than the few interactions that have been identified by various, conflicting studies.

Although we were investigating the role of the C-terminal domain in hPL function, the survey of lysine residues addressed a second issue. A lysine residue was implicated in the catalytic mechanism of porcine PL after inactivating the enzyme by reductive methylation (21). The authors proposed that a lysine residue functioned as the acyl acceptor in the reaction mechanism. Two lysines, Lys81 and Lys296, are near the catalytic site in the crystal structure of hPL but the distance from the nucleophile, Ser153, makes it unlikely that they participate in catalysis. In this study, the mutation of each residue to alanine did not affect the activity of hPL, making the role of either lysine in the active site even more unlikely. Additionally, the mutation of Ser153 in hPL produced inactive mutants, demonstrating the role of this residue in catalysis as the acyl acceptor.

The results reported in this study for the C-terminal deletion mutants are consistent with the contention that the interaction of colipase with PL is more complex than previously believed. The Phe336 and Tyr341 truncated mutants were inhibited by bile salts and reactivated by colipase, demonstrating that a functionally competent interaction of colipase occurred with the N-terminal domain of hPL. Procolipase did bind to the N-terminal domain of hPL in the crystals of the complex formed in the presence of mixed micelles, but contacts with the C-terminal domain were maintained and thought to be important components of the binding reaction (17). The interaction of colipase with the N-terminal domain of lipase is analogous to the binding of the N-terminal domain of lipoprotein lipase with its protein cofactor, ApoCII (22, 23).

Direct measurement of interfacial binding demonstrated that deletion of the C-terminal domain decreased binding, suggesting that this domain is important for binding to the substrate surface. Alternatively, deletion of the C-terminal domain may affect substrate binding sites in the N-terminal domain. Furthermore, the binding was partially restored by the addition of colipase, suggesting that colipase does act to anchor PL to the substrate interface. The observation that colipase incompletely restores binding of the truncated mutant can be explained either by altered interaction of colipase with the mutant or by a decreased contribution of substrate binding sites from the truncated mutant.

To reconcile the available data about the colipase-PL interaction, we propose the following model. The interaction of colipase with PL can occur through two pathways depending on the conditions. Colipase and PL may form a complex in the bulk phase and bind to the substrate interface as a complex. The weak interaction with the PL C-terminal domain keeps colipase associated with PL in a multimeric complex, an association that may start in the zymogen granules and persist after secretion. When the complex binds to the substrate interface, conformational changes permit binding of colipase to the PL N-terminal domain strengthening the association and potentiating lipase activity. Alternatively, colipase may bind to the interface first and anchor PL on the substrate surface. Although the N-terminal domain may be sufficient for PL binding to colipase at the interface, binding to the C-terminal domain may also occur. Colipase interacts with the PL N-terminal domain at the substrate interface in both pathways. It is this interaction that is critical for colipase activation of PL, but binding to the C-terminal domain may strengthen the association of PL and colipase at the substrate interface.

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