Rat GP-3 is a pancreatic lipase with kinetic properties that differ from colipase-dependent pancreatic lipase

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Abstract The pancreas contains three homologous proteins, colipase-dependent pancreatic lipase (PL) and two recently described pancreatic lipase-related proteins, PLRP1 and PLRP2. Rat (r) PLRP2 was first identified as a zymogen granule membrane protein, GP-3. Subsequently, we showed that rPLRP2 could cleave fatty acids from triglycerides, but the kinetic properties of rPLRP2 have not been further investigated. To further characterize rPLRP2, we expressed the recombinant enzyme in a baculovirus system, purified the secreted protein, and measured its kinetic properties. rPLRP2 had a broad pH optimum and the curve was similar to that of rPL. At pH 7.5, rPLRP2 cleaved short, medium, and long chain triglycerides by a kinetic mechanism that did not include interfacial activation. The activity against these substrates was not affected by bile salts. In particular, rPLRP2 did not show the bile salt inhibition typical of PL. Although colipase increased rPLRP2 activity in the presence of bile salts, the increase was only 2- to 5-fold compared to the absolute requirement for colipase that rPL had under these conditions. Finally, rPLRP2 could hydrolyze phospholipids, a substrate poorly hydrolyzed by PL. Our characterization of rPLRP2 demonstrates clear differences among the kinetic properties of rPLRP2 and rPL, rPLRP2, and PLRP2 homologues isolated from guinea pig and coypu pancreas. These findings have important implications for the physiological function of rPLRP2.--**Jennens, M. L., and M. E. Lowe.** Rat GP-3 is a pancreatic lipase with kinetic properties that differ from colipase-dependent pancreatic lipase. *J. Lipid Res.* 1995. **36:** 2374–2382.

Supplementary key words triglycerides • phospholipids • enzyme • protein purification

Pancreatic triglyceride lipase (PL), lipoprotein lipase, and hepatic lipase are members of a lipase gene family that evolved from an ancestral hydrolase. This primordial gene gave rise to a number of enzymes with a common folding pattern, the α/β hydrolase fold (1, 2). Recently, additional members of the lipase gene family were isolated from various sources including mouse cytotoxic T lymphocytes and guinea pig, coypu, human, and rat pancreas (3–7). The predicted amino acid sequences of these proteins and of PL define three lipase family subgroups, PL and pancreatic lipase-related proteins 1 (PLRP1) and 2 (PLRP2).

The kinetic and biological properties of PL are well characterized, but much less is known about the properties of PLRP1 and PLRP2 (8). Even though all three mRNAs are expressed predominantly, if not exclusively, in pancreatic acinar cells, their temporal patterns of mRNA expression differ during development in the rat. Our studies demonstrated that the mRNAs encoding PLRP1 and PLRP2 were maximal after birth whereas the mRNA encoding PL remained low until the suckling–weanling transition (7). The appearance of mRNA for the PLRPs in the suckling period suggested that they may be important for the efficient digestion of fats in the newborn animal.

If the PLRPs play a role in fat digestion, they should be secreted by acinar cells as in PL. The presence of PLRP1 in pancreatic secretions has not been reported and its cellular location is not known. In contrast, the cellular location of PLRP2 has been identified. rPLRP2 was first cloned because it was a zymogen granule membrane protein, GP-3, (9). Subsequently, this same group reported the presence of rPLRP2 in pancreatic secretions where it may function to digest dietary fats (10).

Although the appearance of rPLRP2 in pancreatic secretions is consistent with a role in fat digestion for PLRP2, the association with the zymogen membrane suggests another function for PLRP2. PLRP2 may participate in the fusion of the zymogen membrane with the plasma membrane that must occur prior to the release of

Abbreviations: c, canine; h, human; PL, pancreatic lipase; PLRP, PL-related protein; PI, phosphatidylinositol; r, rat; TDC, taurodeoxycholate.

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zymogen contents. Because the process of exocytosis may require membrane destabilization by unsaturated fatty acids or lysophospholipids, the cleavage of membrane components by lipases may be an initial step in membrane fusion (11, 12).

The potential role of PLRP2 in membrane fusion was supported by studies demonstrating the release of free fatty acids from triolein by rPLRP2 and hPLRP2 (6, 7). At present, there is only indirect evidence that rPLRP2 or hPLRP2 possess phospholipase activity. First, rPLRP2 is highly homologous to two recently described lipases from guinea pig and coypu pancreas (4, 5). Both of these lipases have phospholipase activity and, by analogy, the related rPLRP2 may also have phospholipase activity. Second, zymogen granule membranes have an associated phospholipase activity, which must reside with the membrane-associated proteins like GP-3 (rPLRP2) (13). Although the phospholipase activity was recently attributed to a membrane-bound form of carboxyl ester lipase, the study did not eliminate the possibility that zymogen granule membranes contain more than one phospholipase (14). Its homology to other phospholipases makes rPLRP2 a good candidate to contribute to zymogen granule membrane phospholipase activity. This prediction, which is based solely on sequence homology, may be misleading because the amino acids that confer phospholipase activity in the guinea pig and coypu lipases are unknown. Thus, the determination of phospholipase activity in rPLRP2 can only be made by direct measurement.

To provide additional information about rPLRP2 that will have important implications for its physiological function, we expressed rPLRP2, GP-3, in baculovirus-infected insect cells and characterized the kinetic properties of the purified enzyme. Our data show that rPLRP2 is both a triglyceride lipase and a phospholipase and has several other properties that distinguish it from PL.

METHODS

Expression and purification of rPL and rPLRP2

cDNAs for rPL and rPLRP2 were subcloned into pVL1392 and expressed in insect cells adapted to growth in spinner culture as previously described (7). Three to 4 days post-infection, the medium was harvested by centrifugation at 5000 rpm in a Beckman J2-21 centrifuge with a JA-20 rotor to remove cells and debris.

The clarified medium was dialyzed overnight against 15 volumes of 10 mM Tris-HCl, pH 8.0, and 2 mM benzamidine. Any precipitate was removed by centrifugation and the medium was applied to a DEAE-Blue column equilibrated in the dialysis buffer. rPL appeared in the column pass-through. rPLRP2 bound to the column and was eluted with a 0–0.5 M NaCl gradient in the equilibration buffer. The lipase was located by assay with tributyrin. The pass-through volume containing rPL was adjusted to pH 6.2 with 0.5 M succinate. The fractions containing rPLRP2 were dialyzed against 30 mM Tris-succinate, pH 6.2. Either rPL or rPLRP2 was applied to a CM-Sephadex column equilibrated in 30 mM Tris-succinate. Both were eluted with a 0–0.3 M NaCl gradient. The lipase-containing fractions were located by lipase assay, pooled, and concentrated over an Amicon YM30 membrane. They were then dialyzed against 10 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 21.0 mM $CaCl_2$, and 1 mM EDTA. The purity of the samples was assessed by SDS-PAGE and the protein concentration was determined by the Bradford assay with bovine serum albumin as the standard (15, 16).

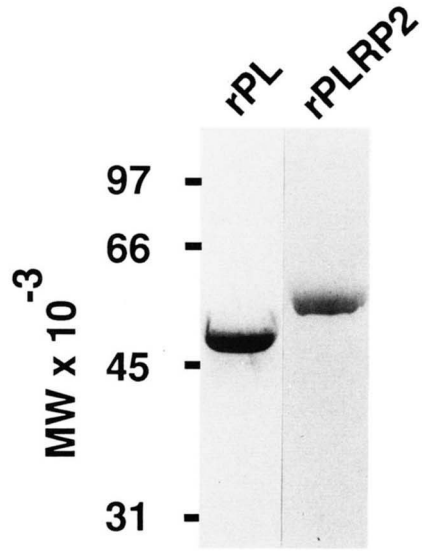

Fig. 1. SDS polyacrylamide gel electrophoresis of recombinant rPL and rPLRP2. Both proteins were expressed in baculovirus-infected insect cells and purified as described in Methods. Ten µg of rPL and 5 µg of rPLRP2 were loaded on a 10% polyacrylamide gel and stained with Coomassie blue. The position of the molecular weight markers is given on the right side of the figure and the sample in each lane is given above the lane.

Human colipase was expressed in baculovirus as the proform and converted to colipase by limited trypsin digestion as described (17). Pig colipase was purchased from Boehringer Mannheim and further purified by immunoaffinity chromatography as described for human colipase. To prepare rat colipase, a rat pancreas was extracted as described by Riley et al. (18) for human pancreas, heated at 60°C for 10 min, centrifuged to remove any precipitate, and dialyzed against 10 mM Tris-Cl, pH 8.0. The sample was applied to a DEAE-

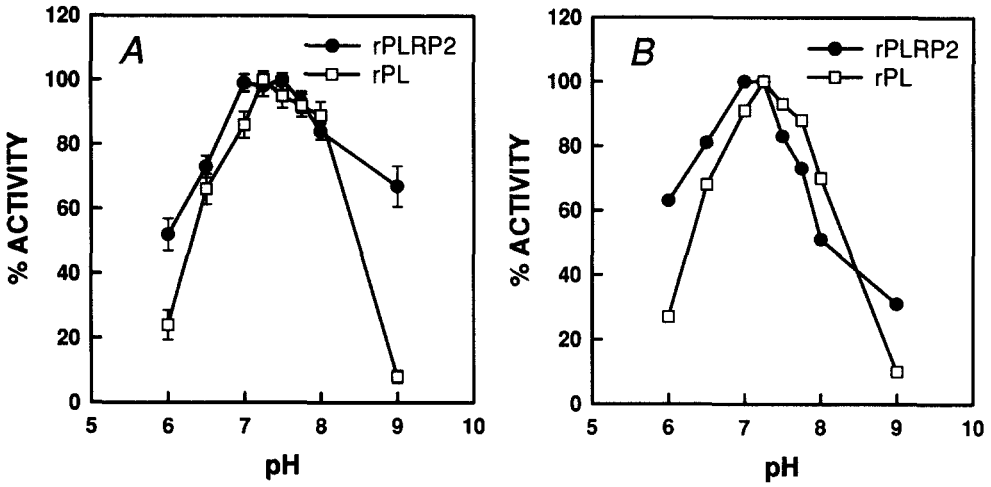

Fig. 2. pH Dependence of rPL and rPLRP2. The activity of each protein against tributyrin was determined as described in Methods. Panel A shows the pH curve in the presence of 4 mM TDC. The values are the mean ± SD of three separate determinations. Panel B shows the pH curve in the absence of TDC. Each value is the average of two separate determinations.

Sephadex column equilibrated in the same buffer and the colipase was eluted with a NaCl gradient from 0.0 to 0.2 M. The fractions containing colipase were pooled and concentrated. The final product had no detectable lipolytic activity.

Lipase activity

Lipase activity was determined potentiometrically in a thermostated reaction vessel at 37°C with a pH-stat (VIT90 Radiometer, Copenhagen). Lipase was added to a mechanically stirred emulsion of substrate in 1.0 mM Tris-Cl, pH 7.5, 0.15 M NaCl, 2.0 mM $CaCl_2$, and 4 mM sodium taurodeoxycholate for the standard assay conditions or 0.3 mM Tris-Cl, pH 7.5, 0.15 M NaCl, 1.4 mM $CaCl_2$ when the bile salt was varied. The substrate emulsion was prepared by adding 0.5 ml of triglyceride to 14.5 ml of buffer and homogenizing. When required, colipase was added in a 2- to 5-fold molar excess. One unit is defined as 1 μmol of fatty acid released/min.

Phospholipase activity

Phospholipase activity was measured by the pH-stat method described by De Haas et al. (19). One egg yolk was homogenized in 100 ml of 4 mM $CaCl_2$, filtered through cheese cloth, and adjusted to pH 8.0. Five ml of this mixture was added to 10 ml of 20 mM deoxycholate to produce the substrate emulsion. One unit is defined as 1 μmol of fatty acid released/min. The [2-^{3}H]phosphatidylinositol assay was done as described (20).

Transfection of COS-1 cells with placental alkaline phosphatase, incubation of the cells with enzymes, and the alkaline phosphatase assay were described previously (21).

RESULTS

Isolation of recombinant rPL and rPLRP2

The pancreas contains several proteins with lipolytic activity that may contaminate preparations of PLRP2 purified from pancreas and could lead to erroneous conclusions about the enzymatic properties of PLRP2. To insure that the PLRP2 used in this study was free of the other lipase family members, we expressed recombinant proteins in a baculovirus system. No detectable triglyceride or phospholipase activity was present in the medium of insect cells infected with baculovirus containing the human colipase gene, demonstrating that no appreciable lipolytic activity was released from these cells (M. E. Lowe, unpublished observations). About 5 mg of rPL or 3 mg of rPLRP2 was purified from 1-L spinner cultures of baculovirus-infected Sf9 cells as described in Methods. Analysis by SDS-PAGE and Coomassie Blue staining showed that each preparation contained a single protein band (**Fig. 1**). rPLRP2 migrated more slowly than rPL indicating a larger molecular size for rPLRP2, 53,000 Da versus 50,000 Da. This result was in agreement with the published size of rat GP-3 (9). The larger size of rPLRP2 compared to rPL is due to a larger size based on the predicted amino acid sequence of the mature proteins, 49,764 Da for rPL versus 50,860 for rPLRP2, and the presence of carbohydrate on rPLRP2 and not on rPL (9 and M. E. Lowe, unpublished results).

pH dependence

The optimum pH for triglyceride hydrolysis is not known for either rPL or rPLRP2. The variation of activity over a range of pH is an important property of enzymes and has implications for function. An acidic pH optimum would argue against an important role in fat digestion and an alkaline pH optimum would argue against a role in membrane fusion because the zymogen granule contents are thought to be acidic. We examined the pH dependence of tributyrin hydrolysis for the two lipases in the presence and absence of 4 mM taurodeoxycholate (TDC) and a 2-fold molar excess of colipase (**Fig. 2A and B**). With TDC present, both rPL and rPLRP2 showed a broad pH optimum between 7 and 8; rPLRP2 was more active at the extremes of the range tested, particularly at alkaline pH (Fig. 2A). In the absence of TDC, the pH optimum for both enzymes was 7.0 to 7.25 and rPLRP2 was less active at more alkaline pH than when TDC was present (Fig. 2B). The similarity of the rPLRP2 curve to the rPL curve suggested that rPLRP2 could function in the duodenal lumen along with rPL.

Interfacial activation

Another important enzymatic property of PL is the marked increase in activity when a water-insoluble substrate presents an oil–water interface. This phenomenon is characteristic of PL family members and has been termed interfacial activation (22). To determine whether interfaces activated rPLRP2, we measured rPLRP2 activity at various concentrations above and below the saturation point of a short chain triglyceride, tributyrin. For comparison, the activity of rPL was also measured. The rPL curve typified interfacial activation with low activity below tributyrin saturation and sharply increased activity above saturation (**Fig. 3**). In contrast, rPLRP2 had high activity at low tributyrin concentrations and its activity increased minimally above saturation, demonstrating that rPLRP2 was not activated by an interface. The slow increase of activity above tributyrin saturation suggested that rPLRP2 may be minimally active against water-insoluble triglycerides.

To determine whether rPLRP2 had activity against insoluble substrates, we measured activity against two triglycerides with no solubility in water, trioctanoin and triolein. The specific activity of rPL and rPLRP2 for each substrate was determined at 4 mM TDC in the presence of colipase (**Table 1**). Although rPL had higher specific activities than rPLRP2 for each substrate, rPLRP2 had readily detectable activity against each substrate, even trioctanoin and triolein. The relative activities of the two lipases for each substrate were quite similar under these assay conditions, suggesting that their substrate preferences were similar. The activity of rPLRP2 against trioctanoin and triolein indicated that rPLRP2 can cleave and interact with water-insoluble substrates.

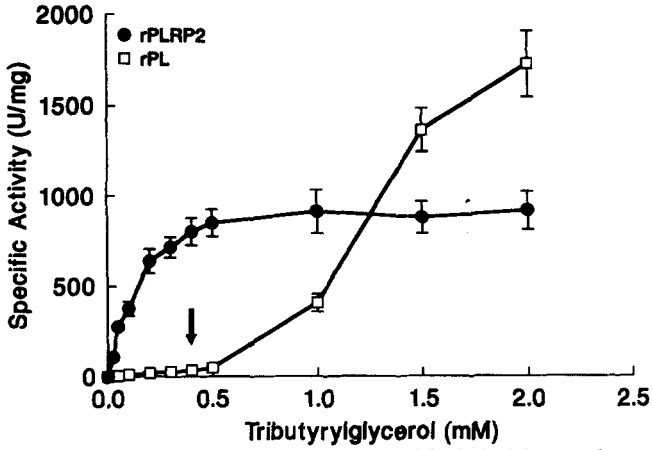

Fig. 3. Interfacial activation of rPL and rPLRP2. Activity was determined at various concentrations of tributyrin as described in Methods. The arrow indicates where saturation occurred as determined by light scattering (26). Values are given as mean ± SD.

Bile salts and colipase

Several well-described conditions influence PL activity (8). Two of these properties are the inhibition of PL by bile salts and the ability of another pancreatic protein, colipase, to restore the bile salt-inhibited activity (23, 24). To investigate the role of bile salts and colipase in lipolysis by rPLRP2, we first determined whether bile salts inhibited this lipase. In the absence of colipase, rPLRP2 had activity against all three triglyceride substrates over a range of TDC concentrations (**Fig. 4 A, B, C**). In distinct contrast to rPLRP2, rPL activity, as expected, was stimulated by low TDC concentrations and almost completely inhibited by higher TDC concentrations (Fig. 4 D,E,F). These findings confirmed that bile salts inhibited recombinant rPL and indicated that bile salts had a minimal affect on rPLRP2.

When we added colipase to the assays, both rPL and rPLRP2 activities increased at all concentrations of bile salt (Fig. 4), but rPL activity was increased to a much greater extent than rPLRP2 activity. rPL depended on colipase for activity at higher TDC concentrations whereas colipase increased rPLRP2 activity only 1.5- to 5-fold. The curves shown in Fig. 4 were obtained with human colipase, but identical results were obtained with rat or porcine colipase or procolipase. These results demonstrated that rPLRP2 did not have an absolute requirement for colipase in the presence of bile salts. This finding is an important distinction between rPLRP2 and rPL that bears on both enzyme mechanisms and physiological functions.

TABLE 1. Activity of rPL and rPLRP2 against triglycerides

	Specific Activity (Relative Activity)		
Lipase	Tributyrin	Trioctanoin	Triolein
rPL	6300(2.1)	4400(1.5)	2940(1.0)
rPLRP2	2140(1.7	1330(1.1)	1240(1.0)

Each enzyme was assayed with 4 mM TDC and a 2-fold molar excess of colipase as described in Methods. Specific activity is U/mg and relative activity is the ratio of specific activities for each substrate to the specific activity of triolein.

Phospholipase activity

Because phospholipase activity has been associated with zymogen granule membranes and rPLRP2 has been associated with these membranes, we determined whether rPLRP2 possessed phospholipase activity (13, 14). We measured the phospholipase activity of rPLRP2 against egg yolk phospholipids, predominantly phosphatidylcholine. rPLRP2 had phospholipase activity that was comparable to the reported specific activity of pancreatic phospholipase A2 (**Table 2**) (9). The rPLRP2 phospholipase activity was maximum at pH 7.0–7.25 and was 40% of maximal activity at pH 8.0 and 6.5 (data not shown). In contrast, rPL had no detectable phospholipase activity in agreement with the results for PLs from other species (8). Thus, phospholipase activity is another crucial difference between the properties of rPLRP2 and rPL that must have a basis in the structural differences of the proteins. Finally, rPLRP2 was responsible for at least part of the phospholipase activity associated with zymogen granule membranes.

Although the egg yolk assay detected phospholipase activity, it did not distinguish among phospholipase A_1, A_2, C, or D activity. Because another abundant zymogen granule membrane protein, GP-2, is anchored by PI-glycan and appears in pancreatic secretions after cleavage of the PI-glycan moiety, the presence of phospholipase C or D activity in rPLRP2 could provide the mechanism for GP-2 release. We tested the hypothesis that rPLRP2 (GP-3) acts to release GP-2 from the zymogen granule membranes by determining whether rPLRP2 could remove a PI-glycan-linked protein, placental alkaline phosphatase, from the cell surface. COS-1 cells were transfected with a cDNA encoding placental alkaline phosphatase. The transfected cells were incubated with either rPLRP2 or phospholipase C. The phospholipase C released 65% of the total cellular alkaline phosphatase activity into the medium, but rPLRP2 did not release alkaline phosphatase from the cell surface. In addition, rPLRP2 had no activity against [2-^{3}H]phosphatidylinositol, a phospholipase C substrate. The inability to demonstrate phospholipase C or D activity in rPLRP2 makes the hypothesis that the function of rPLRP2 is to release PI-glycan-linked zymogen membrane proteins unlikely.

DISCUSSION

Two other lipases with amino acid sequence homology to hPLRP2 and rPLRP2 have been described in guinea pig and coypu pancreas (4, 5). Kinetic studies suggested that the guinea pig and coypu lipases share similar properties including *1)* phospholipase activity, *2)* absent interfacial activation, and *3)* absent colipase effect at high bile salt concentrations (4, 5). In this paper, we show that rPLRP2 shares most but not all of these properties with these other members of the PLRP2 subfamily.

Phospholipase

We demonstrated that rPLRP2 has phospholipase activity making it more likely that phospholipase activity is a general property of the PLRP2 lipase family. Even so, the physiological role of the PLRP2 phospholipase activity remains unclear. In the coypu, no pancreatic phospholipase A_2 activity was found in pancreatic extracts, suggesting that coypu PLRP2 may be responsible for digesting dietary phospholipids in this species (5). A similar conclusion could not be made for the rat or human because pancreatic phospholipase A_2 has been demonstrated in these species.

Another potential role for PLRP2 was suggested by our previous work that demonstrated the maximal expression of the mRNA encoding rPLRP2 after birth (7). This temporal pattern of expression suggested that rPLRP2 may have an important role in the digestion of breast milk fats. Because breast milk fat globules are coated with phospholipids, an enzyme possessing both phospholipase and triglyceride lipase activity may have an advantage in hydrolyzing breast milk fats.

The association of rPLRP2 with zymogen granule membranes suggested yet another possible function for this lipase. If prior digestion of triglycerides and phospholipids is required for membrane fusion, rPLRP2 could participate in the initial steps of membrane fusion. It is properly located and can cleave both triglycerides and phospholipids. At present, it is not known whether other membranes of the PLRP2 family are also tightly bound to zymogen granule membranes and the association may not be a general property of these lipases.

Interfacial activation

Previously, interfacial activation was considered the defining characteristic of lipases, but the demonstration that guinea pig and coypu PLRP2s were not activated by interfaces changed traditional definitions of lipases. We

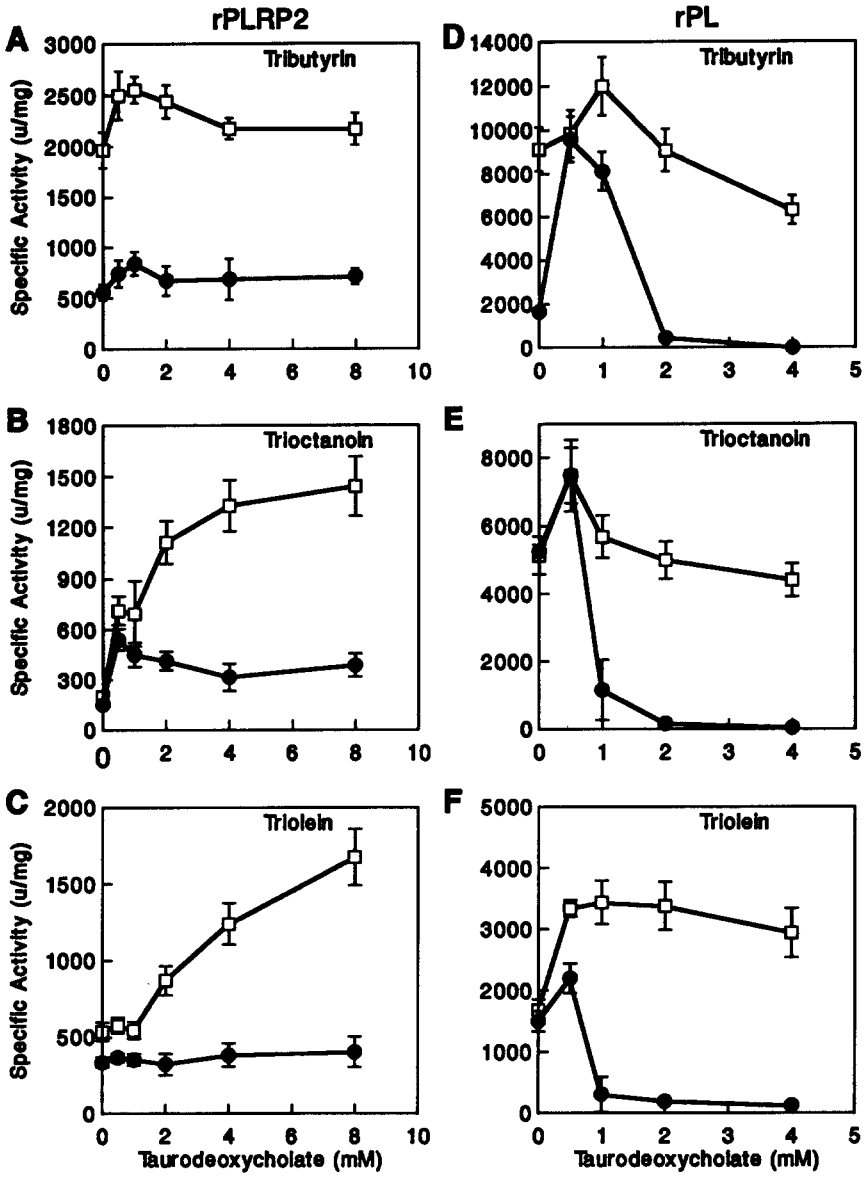

Fig. 4. Colipase and taurodeoxycholate effects on rPL and rPLRP2. Activity was determined against the indicated substrates over a range of TDC concentrations in the absence of colipase (●) and in the presence of colipase (□). The results for rPLRP2 are given in panels A, B, and C. The results for rPL are given in panels D, E, and E. Values are expressed as mean ± SD

demonstrated that rPLRP2 did not require an interface for activity and the presence of an interface did not increase rPLRP2 activity. These findings support the notion that lack of interfacial activation will prove to be a general property of the PLRP2 family.

The mechanism of interfacial activation in PL is thought to be the consequence of a conformational change in a surface loop, the lid domain, that covers the active site in the crystal structure of hPL (25). When crystals of the pig colipase–hPL complex were formed in the presence of mixed micelles, an interface, the lid domain changed conformation to expose the active site. In previous work, we tested this hypothesis by creating hPL mutants with the lid domain deleted (26). These mutants did not show interfacial activation, providing additional evidence that movement of the lid domain may be the physical correlate for the kinetic phenomena of interfacial activation.

Comparison of the coypu and rPLRP2-predicted amino acid sequences with that of rPL showed that they have sequence homologous to the lid domain (**Fig. 5**). In fact, the sequence is quite conserved across species and among members of the lipase gene family. One notable exception is at positions 257 and 258 where arginine and aspartic acid are found in PL, but various divergent residues are found in PLRP2 (Fig. 5). These two residues

TABLE 2. Phospholipase activity of rPL and rPLRP2

Lipase	Activity
	U/mg
rPL	0
rPLRP2	340 ± 28

Activity was measured against egg yolk phospholipids as described in Methods. One unit is defined a 1 μmol of fatty acid released per min. Pancreatic phospholipase A_2 had a specific activity of 700 (19).

interacted with other amino acids in the crystal structure of PL. Arg257 formed a salt bridge with Asp79 in the closed lid conformation and interacted with Tyr268 in the open lid conformation (27). Asp258 interacted with Lys269 in the open lid conformation. These interactions may determine and stabilize conformational changes in the lid that were triggered by an interface. The absence of these interactions may permit the lid domain to spend a significant fraction of time in the open position and allow the free diffusion of water-soluble, monomeric substrate into the active site.

Colipase and bile salts

Guinea pig and coypu PLRP2s were inhibited by micellar concentrations of bile salts, and colipase did not restore activity of either bile salt-inhibited lipase (5). In contrast, rPLRP2 was not inhibited by increasing concentrations of bile salts. This difference between rPLRP2 and the other two PLRP2s may result from variations in the assay systems or reflect true differences in the properties of PLRP2s from different species. If coypu and guinea pig PLRP2s function as lipases in the duodenum, the inhibition by bile salts is counter to efficient fat digestion.

One explanation for these differing results lies in the composition of bile salts in the different species. Guinea pig and coypu have predominantly glycine conjugates whereas rodents have chiefly taurine conjugates. If the lipases have adapted to the types of bile acids secreted by guinea pigs, coypu, and rodents, then assaying these lipases in the presence of TDC may be misleading. Additional extensive studies will be required to define the influence of different bile salt compositions on these enzymes.

Another important difference between the behavior of rPLRP2 and rPL in the presence of bile salts was also apparent from our studies. rPLRP2 was not stimulated by bile salts as was rPL. If the action of bile salts is at the lipid–water interface, then these results suggest that the mechanism of substrate binding and penetration may be markedly different between PL and PLRP2. The behavior of rPLRP2 in the presence of bile salts could not be simply explained by activity against water-soluble monomers because the same phenomenon was also observed with water-insoluble substrates. The lack of bile salt effect may be important in the suckling animal where intraluminal biles salt concentrations are low.

Although colipase increased rPLRP2 activity, the effect was much smaller than seen for rPL. This finding indicated that the interaction of colipase with rPLRP2 differed from the interaction with PL or the other PLRP2s. A

	238																						262
hPL	**C**	K	**K**	**N**	**I**	**L**	**S**	Q	**I**	V	**D**	**I**	D	**G**	I	W	E	**G**	T	R	D	F	**A**	A	**C**
rPL	**C**	Q	**K**	**N**	**I**	**L**	**S**	Q	**I**	V	**D**	**I**	D	**G**	I	W	E	**G**	T	R	D	F	**A**	A	**C**
pPL	**C**	Q	**K**	**N**	**I**	**L**	**S**	Q	**I**	V	**D**	**I**	D	**G**	I	W	E	**G**	T	R	D	F	**V**	A	**C**
hPLRP1	**C**	K	**K**	**N**	**I**	**L**	**S**	Q	**I**	V	**D**	**L**	D	**G**	I	W	A	**G**	T	R	D	F	**V**	A	**C**
rPLRP1	**C**	K	**K**	**N**	**I**	**L**	**S**	Q	**I**	V	**D**	**I**	D	**G**	I	W	S	**G**	T	R	D	F	**V**	A	**C**
cPLRP1	**C**	K	**K**	**N**	**I**	**L**	**S**	Q	**I**	V	**D**	**I**	D	**G**	I	W	E	**G**	T	R	D	F	**V**	A	**C**
hPLRP2	**C**	K	**K**	**N**	**I**	**L**	**S**	T	**I**	T	**D**	**I**	D	**G**	I	W	E	**G**	I	G	G	F	**V**	S	**C**
rPLRP2	**C**	Q	**K**	**N**	**I**	**L**	**S**	T	**I**	V	**D**	**I**	N	**G**	I	W	E	**G**	T	Q	N	F	**V**	A	**C**
mPLRP2	**C**	Q	**K**	**N**	**I**	**L**	**S**	T	**I**	V	**D**	**I**	N	**G**	I	W	E	**G**	T	R	N	F	**A**	A	**C**
COYPU2	**C**	E	**K**	**N**	**I**	**I**	**S**	T	**I**	V	**D**	**V**	N	**G**	F	L	E	**G**	I	T	S	L	**V**	S	**C**

Fig. 5. Comparison of the lid domain amino acid sequences among members of the lipase family. Conserved residues are in bold letters. The residues that differ in the PLRP2 family are underlined. The numbers above the sequences are the positions of the first and last residues of hPL. The references are in the text except for dog PLRP1 (cPLRP1) (29).

comparison of the amino acid sequences of these proteins did not yield a ready explanation for the decreased colipase effects on PLRP2. Most of the human PL residues that interacted with colipase in the crystal structure were conserved. One exception was Tyr404 which was conserved in all PLs but replaced in members of the PLRP2 and PLRP1 families. This finding prompted Thirstrup, Verger, and Carrière (5) to propose that this difference may decrease colipase binding to PLRP2s. We have mutated Tyr404 in human PL and shown that the change made no significant difference in the ability of colipase to restore activity of the mutant human PL. This divergent residue, Tyr404, could not explain the decreased colipase effect on the PLRP2 lipase subfamily (28).

In conclusion, we have expressed and characterized recombinant rPLRP2, another member of the PLRP2 lipase subfamily. The expressed protein has two of the three enzymatic properties that were proposed as general properties of this lipase subfamily. We suggest that only the presence of both triglyceride lipase activity and phospholipase activity and the lack of interfacial activation define PLRP2 lipases. The different behavior in the presence of bile salts between rPLRP2 and the guinea pig and coypu lipases may represent species variations or indicate further subdivisions in the lipase gene family.

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