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Porcine Pancreatic Lipase Related Protein 2 has High Triglyceride Lipase Activity in the Absence of Colipase

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Abstract

Efficient dietary fat digestion is essential for newborns who consume more dietary fat per body weight than at any other time of life. In many mammalian newborns, pancreatic lipase related protein 2 (PLRP2) is the predominant duodenal lipase. Pigs may be an exception since PLRP2 expression has been documented in the intestine but not in the pancreas. Because of the differences in tissue-specific expression, we hypothesized that the kinetic properties of porcine PLRP2 would differ from those of other mammals. To characterize its properties, recombinant porcine PLRP2 was expressed in HEK293T cells and purified to homogeneity. Porcine PLRP2 had activity against tributyrin, triolein and triolein. The activity was not inhibited by bile salts and colipase, which is required for activity of pancreatic triglyceride lipase (PTL), minimally stimulated activity. Similar to PLRP2 from other species, PLRP2 from pigs had activity against galactolipids and phospholipids. Importantly, porcine PLRP2 hydrolyzed a variety of dietary substrates including pasteurized human mother's milk and infant formula and its activity was comparable to that of PTL. In conclusion, porcine PLRP2 has broad substrate specificity and has high triglyceride lipase activity even in the absence of colipase. The data suggest that porcine PLRP2 would be a suitable lipase for inclusion in recombinant preparations for pancreatic enzyme replacement therapy.

Keywords

galactolipid; colipase; lipase; phospholipids; triglycerides

1. Introduction

The efficient digestion of dietary fats is essential for newborns. Infants consume more fat per kg body weight than any other age group, and dietary fats provide 90% of energy for growing tissues [1–3]. In rodents, pancreatic lipase related protein 2 (PLRP2) is critical for dietary fat digestion [4–6]. PLRP2 deficient mice have steatorrhea and poor weight gain until the suckling-weanling transition when pancreatic triglyceride lipase (PTL) expression begins [4]. Human newborns express mRNA encoding PLRP2, but not PTL, in the pancreas at birth suggesting human newborns depend on PLRP2 for dietary fat digestion [6]. Despite the importance of PLRP2 in neonatal nutrition, PLRP2 from various species has

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significantly different enzymatic properties, particularly in regard to substrate specificity and inhibition by bile salt micelles [7–9]. Furthermore, PLRP2 protein was not detected in pancreatic extracts from several species of adult animals including pigs [9]. The other omnivorous animals (humans and rats) tested had easily detectable amounts of PLRP2 in the adult pancreas. A search of DNA databases did not reveal any full-length cDNA encoding PLRP2 isolated from pig pancreas. A single Expressed Sequence Tag for PLRP2 (EW491961) was detected in a pig pancreas library. Full-length PLRP2 cDNA (EU475063.1) has been isolated from pig intestine libraries. The absence of detectable PLRP2 in the pig pancreas and the presence of PLRP2 mRNA in the intestine raise the possibility that porcine PLRP2 may have kinetic properties that differ from those of PLRP2 expressed in the pancreas.

To characterize porcine PLRP2, we cloned the full-length cDNA into a mammalian expression vector and purified recombinant porcine PLRP2 from the medium of transfected HEK293T cells. We then determined the enzymatic properties of porcine PLRP2. Activity and colipase dependence was determined against purified triglycerides, phospholipids, galactolipids and complex emulsions of lipids from pasteurized human mother's milk, cow's milk and infant formula.

2. Materials and Methods

2.1 RT-PCR and molecular cloning

A full length cDNA encoding a porcine PLRP2 clone (clone ID: ITT01_0045_C02) was generously provided by Dr. Hirohide Uenishi at National Institute of Agrobiological Sciences, Ibaraki, Japan. With this cDNA clone serving as template, the full length porcine PLRP2 cDNA was amplified with the Platinum Taq DNA Polymerase High Fidelity kit (Invitrogen) and the following primers: 5'-CAG AAT TCA AAA TGC GCG TCT CTG CTT CAT CCA C-3' (forward) and 5'-GGC TCG AGT TAA TGG TGA TGG TGA TGG TGA CAG GGG TCG AGG GTT TGT AAA AT-3' (reverse). PCR was carried out as following: 94°C × 2 min; 94°C × 30 sec, 55°C × 30 sec, 68°C × 2 min for 15 cycles, and 68°C × 7 min. The entire pig PLRP2 cDNA was directionally subcloned into *EcoR* I/*Xho* I sites of a yeast (pPICZA) and mammalian (pcDNA3) protein expression vector (Invitrogen), respectively. To facilitate the purification of recombinant porcine PLRP2, 6×His tag was fused at the C-terminus. The sequence of all DNA constructs was verified by dideoxynucleotide sequencing analysis.

Snap-frozen pig pancreas and intestine tissue samples (age of 30–120 days) were generously provided by Dr. Aliye Uc (University of Iowa). Total RNA was isolated using TRIzol (Invitrogen) as described by the manufacturer with the following minor modification. One hundred mg of tissue was processed in 2 ml of TRIzol reagent. cDNA was synthesized using M-MLV reverse transcription kit (Ambion). To detect mRNA expression of PLRP2 in pancreas and intestine tissues, PCR was performed as aforementioned and newly synthesized cDNA was used as template. The number of amplification cycles was increased to 30. Purified PCR products were subcloned and their identities were verified by sequencing. PTL and GAPDH were included to serve as controls. The primers were: 5'-GAT GCT GCT AAT CTG GAC A-3' (forward) and 5'-TAG TGA TTA ACA CGG TGT GAG GGT GA-3' (reverse) for PTL; and 5'-TAT AAA TTC CGG CTG CAG CCT TCC CCT GCG CT-3' (forward) and 5'-GTG ATG GCA TGG ACC GTG GTC ATG AGT CC-3' (reverse) for GAPDH.

2.2 Expression of recombinant porcine PLRP2

For expression in yeast, the competent yeast strain *Pichia pastoris* GS115 was transformed by electroporation with Sac I linearized plasmid DNA pPICZA/porcine PLRP2 by

electroporation. Positive colonies were selected as described previously [10]. Media from the methanol-induced cultures were analyzed by immunoblot and lipase activity assay [11–12].

For expression in mammalian cells, HEK293T cells were cultured in DMEM supplemented with 10% FBS. Twenty-four hours prior to transfection, cells were harvested by trypsinization and seeded at 2×10^6 cells in 6-well plates. The cells were transfected with 1.65 μ g of plasmid DNA (pcDNA3 or pcDNA3/porcine PLRP2) using 5 μ l of Fugene 6 (Roche Applied Science) in 100 μ l of Opti-MEM I Reduced Serum Medium (Invitrogen) according to the manufacturer's instructions.

2.3 Western blot analysis

The culture medium and cells from transfected HEK293T cells were harvested 72 hr post transfection. The samples were processed and analyzed by 10% SDS-PAGE as previously described [13]. The separated proteins were transferred onto Immobilon-FL PVDF membranes (Millipore). Membranes were incubated with a rabbit polyclonal antibody against human PTL, followed by a goat anti-rabbit IgG 680 secondary antibody, and the blots were then scanned by Odyssey Infrared Imager (LI-COR). The antibody against human PTL was generated in rabbits against purified recombinant protein prepared in our laboratory and in use since 1992 [14]. Because of the sequence homology between PTL and the related proteins, the antibody has bound PLRP2 from multiple species [5, 13, 15].

2.4 Purification of recombinant porcine PLRP2

To produce recombinant porcine PLRP2, eight 10-cm dishes of HEK293T cells in 10 ml of growth medium were transfected with pcDNA3/porcine PLRP2 plasmid DNA and Fugene 6 when the cells were 70% confluent. Seventy-two hours after transfection, conditioned media were collected. The pooled culture media was incubated with 3 ml of pretreated ProBond Resin (Invitrogen) overnight with gentle agitation on a rocking platform in a cold room. The mixture was loaded onto a 15 ml Econo-Pac Disposable Chromatography Column (Bio-Rad) and drained by gravity. The column was further washed with 50 ml of washing buffer-1 (50 mM Na_xPO_4 , 500 mM NaCl, 10 mM imidazole, pH 8.0). Finally, the bound protein was eluted with 10 ml of elution buffer-1 (50 mM Na_xPO_4 , 500 mM NaCl, 300 mM imidazole, pH 8.0).

The eluted protein sample was first dialyzed against washing buffer-2 (50 mM Na_xPO_4 , 150 mM NaCl, 10 mM imidazole, pH 7.4). One 1-ml HiTrap Chelating HP column (GE Healthcare) was charged with Ni_2SO_4 and equilibrated with washing buffer-2. The dialyzed sample was then loaded onto the equilibrated column using an AktaExplorer Chromatography system (Amersham Biosciences). The bound recombinant protein was eluted from the column using a linear concentration gradient from 10 to 300 mM imidazole within 60 min at a rate of 1 ml/min. Fractions containing recombinant protein were identified by optical density at 280 nm and lipase activity assay. The lipase activity eluted in about 10 fractions. The three fractions with the highest activity were pooled and concentrated over an Amicon Ultra-15 30K MWCO centrifuge filter (Millipore). The buffer was exchanged to washing buffer-2. The protein concentration was determined by UV light spectrophotometry at 280 nm using an extinction coefficient of $E^{1\text{cm}}_{1\%} = 1.35$.

2.5 Determination of protein integrity

The purified proteins were analyzed by gel staining to confirm their homogeneity and integrity. Briefly, varying amounts of protein were resolved by 10% SDS-PAGE along with purified recombinant human PTL protein and stained with GelCode Blue Stain Reagent

(Pierce) [12]. The gel staining was scanned with a Molecular Imager VersaDoc MP 4000 System (Bio-Rad).

2.6 Lipase activity assay

Triglyceride lipase activity was determined at 25°C by the pH-stat method as previously described using 2 µg of lipase [16–17]. Unless otherwise stated, the reaction was carried out for 5 min in the presence or absence of a 5-fold molar excess of recombinant human colipase (2 µg) at the indicated sodium taurodeoxycholate (NaTDC) concentration. Activity was determined from the linear portion of the titration curve. One unit (U) equals 1 µmole fatty acid released/min. Lipase activity against Intralipid, pasteurized mother's breast milk (heat treatment at 73 °C for 20 minutes to inactivate bile salt stimulated lipase), whole cow's milk (3.4 % fat) and infant formula (Similac Advance Ready-To-Feed formula, 3.3% fat, Abbott Nutrition) was assayed for the duration of 30 min. For these assays, 3 mL of substrate and 12 mL reaction buffer (1 mM Tris-Cl, pH 8.0, 2.0 mM CaCl₂, and 0.15 M NaCl) and a bile salt mixture were emulsified in a Dounce homogenizer. In these experiments, a 200 mM mixture containing 84 mM of taurocholate, 44 mM of glycocholate, 52 mM of taurochenodeoxycholate and 20 mM of glycochenodeoxycholate was added to a final concentration of 4 mM [14, 18–19]. As with the purified substrates, activity was determined from the linear portion of the curve.

Galactolipase and phospholipase activities were determined at 25°C using the pH-stat method over 5 min [15]. For each galactolipase activity assay, 15 mg of digalactosyldiacylglyceride (Larodan AB, Malmo Sweden) was dispersed in 15 ml of 4 mM NaTDC assay buffer and 5 µg of porcine PLRP2 or 3 µg human PLRP2 was added. Phospholipase activity was measured following the methods described previously [20]. For each assay, 200 mg of egg yolk phosphatidylcholine (Sigma) was dispersed in assay buffer containing 1 mM Tris-HCl (pH 8.0), 100 mM NaCl, 3 mM CaCl₂, and 13.3 mM deoxycholate, and 15 µg of porcine PLRP2 was included.

2.7 Colipase titration

The interactions of porcine PLRP2 with colipase were assayed by adapting the method described previously [21]. The assays were performed by measuring lipase activity over a range of colipase concentration (0–53 nM) with a constant concentration of lipase (2.6 nM) and excess substrate (0.2 ml of triolein) in 4 mM NaTDC assay buffer in a total volume of 15 ml.

3. Results

3.1 Characterization of porcine PLRP2 amino acid structure

Dideoxynucleotide sequencing of the final PCR products revealed identical sequences for the cDNA prepared from the cDNA clone provided by Dr. Uenishi and from the cDNA prepared from pancreatic and intestinal mRNA. Each of the sequences matched one another and the sequence deposited in Genbank (ACI00230.1) with 100% identity. The cDNA sequence predicted a protein of 471 amino acids including a 17 amino acid signal peptide. Human PLRP2 has a predicted amino acid sequence of 469 amino acids including a 17 amino acid signal peptide. The difference is accounted for by the presence of two additional glutamic acid residues at positions 313 and 314 (Fig 1). The predicted molecular weights for the pig and human lipases are 51,335 and 50,113 Dal, respectively. Porcine PTL has a predicted molecular weight of 49,669 Dal. The predicted amino acid sequence of porcine PLRP2 is 78% identical and 86% homologous to the sequence of human PLRP2 and 62% identical to both porcine and human PTL (Fig 1).

3.2 Expression and purification of porcine PLRP2

We initially attempted to express recombinant porcine PLRP2 protein in *Pichia pastoris* and cloned a full-length cDNA into a yeast expression vector. After transformation of yeast, we were unable to identify any clones that secreted appreciable amounts of porcine PLRP2. We next cloned the cDNA into a mammalian expression vector and transfected HEK293T cells. After transfection, we performed activity assays and immunoblot of the medium. The medium contained activity against all three triglyceride substrates even in the absence of colipase (Table 1), whereas the activity of the medium from mock transfection was undetectable. The addition of colipase to the assay stimulated activity. Immunoblot analysis revealed a predominant band in both the medium and cell lysate from HEK 293T cells transfected only (Fig 2A.). We estimated that the medium contained 20–40 µg/ml of porcine PLRP2 with a specific activity against tributyrin of 1.1 ± 0.33 U/mg protein. We purified porcine PLRP2 from 80 ml of medium with a final yield of ~1.1 mg. The purified lipase had a specific activity against tributyrin of 3500 ± 105 U/mg. Analysis of purified porcine PLRP2 by SDS-PAGE and GelCode Blue staining revealed a single band that migrated above human PTL as expected from the predicted molecular weight (Fig 2B.).

3.3 Triglyceride lipase activity of porcine PLRP2

We first determined whether bile salt would inhibit porcine PLRP2 and whether colipase was required for activity in the presence of bile salts. Assays were done with tributyrin, trioctanoin and triolein emulsified in various concentrations of NaTDC (Fig 3.). The assays were done without colipase and with a 5-fold molar ratio of colipase to porcine PLRP2. Increasing NaTDC concentration from 0 mM NaTDC to 4 mM NaTDC decreased activity against tributyrin by about 30% (Fig 3A.). Bile salt concentration had no effect on activity against trioctanoin (Fig 3B.). Activity against triolein increased linearly with NaTDC concentration with the highest activity observed at 4 mM NaTDC (Fig 3C.). The addition of colipase increased the activity 2-fold or less depending on the substrate.

3.4 Colipase dependence of porcine PLRP2

To better characterize the interaction with colipase, we measured the ability of colipase to increase porcine PLRP2 activity over a range of colipase concentrations in the presence of excess triolein and a constant concentration of lipase (Fig 4.). We compared the behavior of porcine PLRP2 to that of human PTL. From the activity curves we determined the ratio of colipase to lipase at half-maximal activity for both lipases. Although the value for porcine PLRP2 (0.61 ± 0.17) was lower than for human PTL (2.1 ± 0.78), the difference was not statistically significant ($P = 0.132$). The activity of porcine PLRP2 saturated with increasing concentrations of colipase.

To further investigate the interaction between porcine PLRP2 and colipase relative to the interaction of PTL and colipase, we compared the ability of porcine PLRP2 and human PTL to compete with human PTL-S153G, an inactive form of PTL, for binding with colipase [22]. PTL-S153G interacts with lipids and colipase with the same affinity as PTL [23]. Activity was determined against triolein emulsified in 4 mM NaTDC. Colipase, PTL or porcine PLRP2 and PTL-S153G were added in a molar ratio of 0.5:1.0:1.0. As expected, the activity of human PTL was decreased to 47% by PTL-S153G reflecting the similar affinity of the two lipases for colipase (Table 2). In contrast, porcine PLRP2 retained about 90% of its activity in the presence of PTL-S153G.

3.4 Phospholipase and galactolipase activity of porcine PLRP2

Because other PLRP2 lipases have activity against phospholipids and galactolipids, we measured the activity of porcine PLRP2 against those substrates. Porcine PLRP2 had

activity against digalactosyldiglyceride, 39.1 ± 3.9 U/mg compared to 164 ± 14.1 U/mg for human PLRP2. The porcine PLRP2 also had activity against phosphatidylcholine, 22.9 ± 3.6 U/mg. This activity is similar to the activity of human PLRP2 [13].

3.5 Porcine PLRP2 activity against dietary lipids

The high activity against triglycerides of porcine PLRP2 in the presence of bile salts and absence of colipase coupled with its ability to hydrolyze galactolipids and phospholipids makes porcine PLRP2 a reasonable candidate for the lipase in a preparation of recombinant digestive enzymes to treat pancreatic insufficiency. Thus, we investigated whether porcine PLRP2 had activity against a variety of more complex substrates including Intralipid, whole cow's milk, pasteurized human mother's milk and infant formula. The assays, done with and without colipase, were compared to the activity of human PTL (Fig 5.). Porcine PLRP2 had activity against all of the substrates in the absence of colipase and colipase increased the activity with all substrates. The largest increase, about 2-fold, was observed with Intralipid. PTL also had activity against all substrates but only in the presence of colipase. The activities of porcine PLRP2 without colipase were indistinguishable from the activities of human PTL and colipase with Intralipid and whole cow's milk. In the presence of colipase, the activities of porcine PLRP2 against Intralipid and whole cow's milk were higher than the activities of PTL with colipase. Without colipase, the activity of porcine PLRP2 against human mother's milk and infant formula was about 30% less than that of PTL with colipase. With colipase, the activities of porcine PLRP2 against human mother's milk and infant formula were identical to activities of human PTL with colipase.

3.6 Expression of mRNA encoding PLRP2 in pig pancreas and intestine

Because PLRP2 protein was not detected in pig pancreas and the only full-length cDNAs have been isolated from pig intestinal libraries, we determined whether mRNA encoding porcine PLRP2 could be detected in RNA isolated from pig pancreas. To accomplish this goal, we performed RT-PCR on total RNA isolated from pig pancreas and intestine using primers specific for porcine PLRP2 and PTL (pancreas-specific tissue expression) and confirmed their identities by sequencing. mRNA encoding PLRP2 was detected in both tissues whereas mRNA encoding PTL was only detected in pancreas suggesting the expression of porcine PLRP2 and PTL exhibit distinctive tissue distribution patterns (Fig 6.).

4. Discussion

PLRP2 lipases have been identified in multiple species, primarily herbivores and omnivores [9]. Many have been expressed, purified and characterized for their catalytic properties. Important differences in the kinetics, substrate specificity, bile salt inhibition and colipase dependence have been reported. All PLRP2 lipases have activity against triglycerides, although the reported specific activities vary as much as 10-fold, even when done in the same laboratory [9, 13, 15, 24–27]. Porcine PLRP2 is no exception. It has similar activity to mouse and rat PLRP2 and almost 10-fold higher activity than human PLRP2 against tributyrin, trioctanoin and triolein [13, 15, 26].

As with rat and mouse PLRP2, porcine PLRP2 activity against triglycerides is not inhibited by bile salts. Bile salts cause a small decrease in activity with tributyrin and no change in activity against trioctanoin. In contrast, activity against triolein increases with increasing NaTDC concentration suggesting this bile salt improves triolein emulsification. Colipase increases activity at all concentrations of bile salt with rat, mouse and porcine PLRP2. The amount of stimulation varies with the substrate and species. The increase varies from 1.5 to 5-fold for rat and mouse PLRP2 and no more than 2-fold with the porcine PLRP2. Thus,

porcine PLRP2 activity is less dependent on colipase than rat and mouse PLRP2. In contrast, human, horse and coypu PLRP2 triglyceride lipase activities are inhibited by increasing concentrations of bile salts [13, 24–25, 27]. Of these lipases, colipase increases activity of human PLRP2 and has little to no effect on the activity of horse or coypu PLRP2.

Interestingly, porcine PLRP2 activity was minimally inhibited by S153G PTL, which has the same affinity for colipase as does PTL. This result may be interpreted two ways. Either porcine PLRP2 has significantly higher affinity for colipase than does PTL or porcine PLRP2 does not require an interaction with colipase for activity. The colipase titration curve demonstrates that porcine PLRP2 does not have higher affinity for colipase than does PTL. Thus, the likely explanation for our results is that porcine PLRP2 has weak or no direct interactions with colipase. The modest influence of colipase on porcine PLRP2 activity may result from modification of the substrate's physical properties by colipase as demonstrated in monolayer studies [28–29].

All of the PLRP2 lipases have activity against galactolipids [9, 15, 30–32]. Most have activity against both mono- and digalactosyldiacylglycerides. Rabbit PLRP2 had low activity against monogalactosyldiacylglyceride and no detectable activity against digalactosyldiacylglyceride [9]. Porcine PLRP2 can hydrolyze digalactosyldiacylglyceride. We did not test activity against monogalactosyldiacylglyceride. Galactolipids are abundant in all green plants, fruits, roots, seeds and tubers. They are ingested in large quantities by herbivores and to a lesser extent by omnivores. Because PLRP2 was identified in the pancreas of non-ruminant herbivores and omnivores but not in the pancreas of ruminant herbivores and carnivores, it was suggested that the main role of PLRP2 in adults is as a galactolipase [9, 32]. Pigs may represent an exception. They are omnivores but may not synthesize and secrete PLRP2 from the pancreas.

PLRP2 lipases also have activity against phospholipids but the range of specific activities varies considerably. Coypu and rabbit have relatively high phospholipase activity [9, 27]. Human, pig, horse, rat and mouse have lower phospholipase activity [9, 25, 27, 33]. Some of the variation may depend on the type of phospholipid used in the assay, most often phosphatidylcholine. Coypu and rat PLRP2 have a clear preference for phosphatidylethanolamine and phosphatidylglycerol over phosphatidylcholine [33]. Human PLRP2 has similar activity against all three [32]. Horse, porcine and mouse PLRP2 lipases have only been tested with phosphatidylcholine.

We also tested the activity of porcine PLRP2 against more complex substrates, Intralipid, pasteurized human mother's milk, whole cow's milk and an infant formula. Berton et al had previously shown that human PLRP2 had activity against cow's milk fat globules, but no other PLRP2 lipases or substrates have been tested [34]. Of note, porcine PLRP2 had activity against all of the substrates. The activity was comparable to the activity of the PTL-colipase complex even when colipase was not included in the assay with porcine PLRP2. This finding is significant because the PTL-colipase complex generally has much higher activity against these substrates than any other lipase.

In this study, we detected mRNA encoding PLRP2 in pig pancreas, which suggests that the pig pancreas expresses PLRP2. There are several possible explanations why the earlier study failed to identify PLRP2 in pig pancreas [9]. The mRNA may not be translated. The protein may be present at low levels. Or, the protein may be lost during purification as we experienced with rat PLRP2 (Lowe, ME, unpublished results). The same may occur with porcine PLRP2. Nothing is known about expression of PLRP2 in pig pancreas. Since studies in pigs have only been done in adults, it remains possible that PLRP2 is expressed and

secreted from the pancreas of piglets. Until PLRP2 protein is identified in pig tissues or secretions, its role in the pig remains uncertain.

In conclusion, our study demonstrates that the catalytic properties of porcine PLRP2 are similar to those described for mouse and rat PLRP2. More importantly, we found that porcine PLRP2 has high activity against dietary lipids even in the absence of colipase and presence of bile salt micelles. The findings suggest that porcine PLRP2 can function in the duodenum to digest dietary fats. Whether it has that role in the pig was not addressed in these studies. Even so, the properties of porcine PLRP2 raise the possibility that the enzyme could function as a component of recombinant pancreatic enzyme therapy for patients with pancreatic insufficiency or newborns who are homozygous for a loss-of-function mutation in PLRP2.

Acknowledgments

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Abbreviations used

PLRP2	Pancreatic lipase related protein 2
PTL	Pancreatic triglyceride lipase
NaTDC	Sodium taurodeoxycholate

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HIGHLIGHTS

- Porcine PLRP2 has high colipase-independent triglyceride lipase activity in the presence of bile salts
- Porcine PLRP2 has galactolipase and phospholipase activity
- Porcine PLRP2 has comparable activity to human PTL against human mother's milk and infant formula
- mRNA encoding porcine PLRP2 is detectable in the pancreas and small intestine

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PPLRP2 1 MLPSWTIGLLLLATVRGKEHCYOPFGCFSDETFWARTCHWPFKLFPWAKDKIDTFLFLYT
HPLRP2 1 MLPPWTIGLLLLATVRGKEVCYQOLGCFSDERKFWAGTLQRPVLLLPWSPEDIDTFLFLYT
PPTL 1 MLLIWTISLL-LGAVLGGSEVCFRLGCFSDDAFWAGIVQRPKLILPWDRKDVNTRFLFLYT
HPTL 1 MLDLWTISLL-LGAVAGKEVCYERLGCFSDDSPWSCITERPLHILPWSFKDVNTRFLFLYT

PPLRP2 61 NENPNNFQLINITNLDTIASNFOLDKTRFIIHGFIIDKGEDSWPSEMCKKMFVEKYNVC
HPLRP2 61 NENPNNFQLITGTEPPTIASNFOLDKTRFIIHGFIIDKAEDESWPSEMCKKMFVEKYNVC
PPTL 60 NENQDNVQELV-ADPSTITDSNFRMDKTRFIIHGFIIDKGEDSWLSEKCNLFKVESYNVC
HPTL 60 NENPNNFQEVA-ADSSSISGSNFKTRKTRFIIHGFIIDKGENWLANVCKNLFKVESYNVC

PPLRP2 121 ICVDWRRGALTRYTOAVHNTIRVGAETIAFLIOGLSTKFDYNPENVEHLIGHSLGAETAAEA
HPLRP2 121 ICVDWRHSGSRAMYTOAVONIRVGAETIAFLIOALSTOLGYSLEDVHVIGHSLGAETAAEA
PPTL 119 ICVDWKGGSRTGYTOASONIRVGAETIAFLIOALSTOLGYSLEDVHVIGHSLGSAAGAAEA
HPTL 119 ICVDWKGGSRTGYTOASONIRVGAETIAFLIOALSTOLGYSLEDVHVIGHSLGSAAGAAEA

PPLRP2 181 GRLGCHVGRLTGLDPAOPCFONTPEEVRLDPSDAMFVDVIHTDSAPFIPLGFGMSQKV
HPLRP2 181 GRLGCHVGRLTGLDPAOPCFODEPEEVRLDPSDAMFVDVIHTDSAPFVPLGFGMSQKV
PPTL 179 GRRTNGTIGRITGLDPAEPCFOGTPELVRLDPSDAKFVDVIHTDAPFIPLGFGMSQKV
HPTL 179 GRRTNGTIGRITGLDPAEPCFOGTPELVRLDPSDAKFVDVIHTDAPFIPLGFGMSQKV

PPLRP2 241 GHLDFPFGGKEMPQCKNLTSTIVDIDGIWEGIEDFAACNHLRSYKYYSIFSPDGFL
HPLRP2 241 GHLDFPFGGKEMPQCKNLTSTIVDIDGIWEGIGGFVACNHLRSYKYYSIFSPDGFL
PPTL 239 GHLDFPFGGKEMPQCKNLTSTIVDIDGIWEGTRDFVACNHLRSYKYYSIFSPDGFL
HPTL 239 GHLDFPFGGKEMPQCKNLTSTIVDIDGIWEGTRDFVACNHLRSYKYYSIFSPDGFL

PPLRP2 301 GYPCASYDEEQEENKCFPCFARGCCKMGHYADOFQKTSVAVGQTFFLNTGDSGNFTRWRR
HPLRP2 301 GYPCASYDEEQE--SKCFPCFARGCCKMGHYADOFQKTSVAVGQTFFLNTGDSGNFTSWRR
PPTL 299 GFPCDSYNNVETA--NKCFCPCFARGCCKMGHYADRFPGKTNGVSOVFYFLNTGDASNFAFRR
HPTL 299 GFPCASYNNVETA--NKCFCPCFARGCCKMGHYADRFPGKTNDVGQKFYFLNTGDASNFAFRR

PPLRP2 361 YRVSVTLGAKRNVHGVIIRIALYGSNANSKQYEIFKGSLOPNARYTHDIDVDLNVCKVQKV
HPLRP2 359 YKVSVTLSGKEKNGYIRIALYGSNANSKQYEIFKGSLOPDASHTCAIDVDNVCKIQKV
PPTL 357 YKVSVTLSGKK-VTGHILVSLFGNENGSQYEIFKGTLOPDNTHSNEFSDVDVGDQLQKV
HPTL 357 YKVSVTLSGKK-VTGHILVSLFGNENGSQYEIFKGTLOPDSTHSNEFSDVDVGDQLQKV

PPLRP2 421 KFLWYNHITDLFHPGLGASQVMVOSGEDKTEHKFCSSDTVRENILQTLNFC
HPLRP2 419 KFLWNRGINLSEPKLGASQITVOSGEDKTEYNFCSSDTVRENVLQSLYPC
PPTL 416 KFLWYNNVINPTLPRVGASKITVER-NDCKVYDFCSQETVREEVLLTLTPC
HPTL 416 KFLWYNNVINPTLPRVGASKITVET-NVCKQPNFCSPETVREEVLLTLTPC

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Fig 1.

Amino acid sequence alignment of porcine and human PLRP2 and PTL. The amino acid sequence was predicted from the nucleotide sequence of our cDNA clones for pig PLRP2. The sequences for porcine PTL (NP_000927.1), human PLRP2 (CAG33230.1) and human PTL (NP_000927.1) were obtained from data in the NCBI protein database. Alignment was performed with Clustal Omega [35–36]. Boxes were added with Boxshade.

Fig 2.

Expression in HEK293T cells and purification of porcine PLRP2. A. HEK293T cells were transfected with either empty vector (Mock) or a vector containing cDNA encoding porcine PLRP2 as described in Methods. The medium and cells were harvested 72 hr after transfection and prepared protein samples were subjected to 10% SDS-PAGE and detected by immunoblot. B. Porcine PLRP2 was purified as described in Materials and Methods and analyzed by SDS-PAGE with GelCode Blue staining. For comparison purified human PTL (hPTL) was included on the gel. Human PTL was purified as previously published [12]. Five μ g of each lipase was loaded onto the gel.

Fig 3.

Activity of porcine PLRP2 against various triglyceride substrates in the presence of NaTDC with and without colipase. A. Activity against tributyrin was determined in the pH-stat. B. Activity against trioctanoin was determined in the pH-stat. C. Activity against triolein was determined in the pH-stat. For each substrate the mean $\pm$ SD ($n = 3$) are plotted. U = μ moles fatty acid released/min. Closed circles, no colipase; Open circles, 5-fold molar excess of human colipase purified as previously described [10].

Fig 4.

Colipase dependence of porcine PLRP2 compared to human PTL. Activity against triolein emulsified in 4 mM NaTDC was determined in the pH stat using increasing concentrations of human colipase. U = μ moles fatty acid released/min. The mean $\pm$ SD (n = 4) are plotted. Open circles, human PTL; Black triangles, porcine PLRP2.

Fig 5.

Activity of porcine PLRP2 against various physiological substrates compared to human PTL. Activity against lipids in Intralipid, pasteurized human mother's milk, whole cow's milk and infant formula was determined in the pH-stat for 30 min in the presence of 4 mM NaTDC with and without a 5 molar excess of human colipase. U = μ moles fatty acid released/min. For each substrate the mean $\pm$ SD (n = 3) are plotted. White bars, porcine PLRP2 with colipase; Black bars, porcine PLRP2; Gray bars, human PTL with colipase, Hatched bars, human PTL.

Fig 6.

RT-PCR of mRNA from pig pancreas and intestine with primers specific for porcine PTL or PLRP2. The reaction was done as described in Materials and Methods. The products were separated by agarose gel electrophoresis and the products identified by ethidium bromide staining. The lanes for PTL and PLRP2 are labeled. The source of the mRNA is indicated below each lane. The products for PLRP2 and PTL migrate at ~ 1500 bp. Glyceraldehyde 3 phosphate dehydrogenase (GAPDH) served as an endogenous control. Pan, pancreas; Int, intestine; M, 1000 bp markers.

Table 1Lipase activity in medium from transfected HEK293T cells^a

Lipid	Activity (nmoles FA/min)	
	No Colipase	Colipase
Tributyrin	1818 ± 74	4423 ± 114
Trioctanoin	2141 ± 74	3933 ± 100
Triolein	639 ± 51	1510 ± 59

^a Activity was determined in the pH-stat using lipid emulsified in 4 mM NaTDC. The assay included 3 µg of colipase and 50 µl of medium. The values are the mean ± SD of three separate transfections.

Table 2Affinity of porcine PLRP2 for colipase by competition assay with S153G PTL^a

Lipase	Activity (U/mg)		Residual Lipase Activity (%)
	No colipase	With colipase	
		No S153G With S153G	
Porcine PLRP2	1907 ± 28.5	2707 ± 48.0 2426 ± 80.7	90
Human PTL	0.0	2095 ± 140 995 ± 154	47

Each assay was done by the pH-stat method with triolein emulsified in 4 mM NaTDC. Each assay contained 3 µg of each lipase as indicated with or without 0.3 µg of human colipase. U = µmoles fatty acid released/min. Residual lipase activity = (Activity with colipase and S153G/Activity with colipase and no S153G) × 100. Each value is the mean ± SD of three determinations.