Pancreatic Lipase-related Protein 2 Is the Major Colipase-Dependent Pancreatic Lipase in Suckling Mice[1]

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Abstract

Suckling mice express colipase before the expression of pancreatic triglyceride lipase. Yet, efficient fat digestion in newborns requires colipase, suggesting that colipase may act as a cofactor for another lipase such as pancreatic lipase-related protein 2 (PLRP2). We determined whether PLRP2 or another lipase depends on colipase for maximal activity in newborn mice by analyzing extracts from the pancreas of 4-d-old colipase-deficient and PLRP2-deficient mice. Pancreatic extracts from colipase-deficient pups had lipase activity that was stimulated onefold by the addition of exogenous colipase ($P < 0.001$). The activity was completely inhibited by an antibody against pancreatic triglyceride lipase that also recognizes PLRP2. In contrast, pancreatic extracts from PLRP2-deficient pups had significantly lower baseline activity and no colipase-dependent activity. The baseline activity was not inhibited by the anti-pancreatic triglyceride lipase antibody or an antibody against carboxyl ester lipase. We next separated the extracts into two fractions, one containing PLRP2 and the other devoid of PLRP2. All of the colipase-dependent activity segregated with the PLRP2-containing fraction, consistent with the conclusion that PLRP2 is the major colipase-dependent lipase in the pancreas of newborns.

Keywords

colipase; dietary fat; newborns; nutrition

Dietary triglycerides, the predominant dietary fat, play an important role in nutrition. They provide a major energy source, they are precursors for cellular membranes and for prostaglandins, thromboxanes and leukotrienes, a vehicle for fat-soluble vitamins, and they improve the palatability of foods (1). Dietary fats are particularly important for newborn infants who consume more dietary fat than at any other time in their life (2). In addition to the benefits listed above, dietary fats contribute ~90% of the energy retained in new tissues during the first 6 mo of life (2,3). Consequently, the development of newborn infants depends on the efficient digestion of dietary triglycerides, which must be cleaved into fatty acids and monoacylglycerols before absorption (4,5).

Adult humans and mice require pancreatic triglyceride lipase and colipase for efficient dietary fat digestion, but the situation differs in newborns because pancreatic triglyceride lipase does not contribute to dietary fat digestion in newborns (6–8). Although pancreatic triglyceride lipase and colipase function together as a complex, they have distinctly different temporal patterns of expression (9,10). Newborns and suckling infants express colipase, but do not express pancreatic triglyceride lipase until the suckling-weanling transition. Even so, colipase

has a critical role in dietary fat digestion in newborns as evidenced by a study showing that mice with colipase deficiency malabsorb dietary fats at an age when pancreatic triglyceride lipase could not be detected (7). One explanation for this finding is that colipase interacts with another lipase in newborns.

A potential candidate to interact with colipase is pancreatic lipase-related protein 2 (PLRP2), a homologue of pancreatic triglyceride lipase. Several studies of PLRP2 clearly demonstrate the contribution of this lipase to dietary fat digestion in newborns. PLRP2 possesses triglyceride lipase activity and newborns of many species express mRNA encoding PLRP2 from birth (11). Importantly, PLRP2-deficient pups have steatorrhea and their feces contain large quantities of incompletely digested dietary fats (12). In vitro, colipase increases the activity of recombinant human, mouse and rat PLRP2, but bile salt micelles do not completely inhibit these lipases, and colipase is not necessary for activity as seen with pancreatic triglyceride lipase (9,12,13). In this study, we address the hypothesis that PLRP2 is the predominant, if not only colipase-dependent lipase in the pancreas of newborns.

METHODS

Animals

Both procolipase and PLRP2-deficient mice were used in this study (7,12). Animal care and use were in accordance with institutional Animal Studies Committee guidelines and our animal protocol was approved by the institutional Animal Studies Committee. Mice consumed PicoLab 5053 rodent diet (Purina Mills, Richmond, IN) and water ad libitum. The mice were killed by anesthesia with Avertin (15 mg/kg) and cervical dislocation.

Protein methods

Extracts were made of whole pancreas from 4-d-old pups. After removal, two glands were sonicated for 10 s in 200 μL of extraction buffer [50 mmol/L Tris-Cl containing one Mini-Complete tablet per 10 mL (Roche Molecular Biochemicals, Manheim, Germany)]. An aliquot was removed for assay before centrifugation at $14,000 \times g$ for 10 min at 4°C. The supernatant was harvested for further analysis. Colipase was removed from the extracts of the pancreata of PLRP2-deficient and wild-type mice by dialysis. We demonstrated the efficient removal of colipase by adding an aliquot of the heat-inactivated extract to a reaction mixture of pancreatic triglyceride lipase and inhibitory concentrations of taurodeoxycholate as described below. The extract did not restore activity to bile salt–inhibited pancreatic triglyceride lipase, showing that the colipase had been effectively removed. Protein (1 mg) was assayed for lipase activity against trioctanoin emulsified in 4 mmol/L taurodeoxycholate by the pH-stat method as previously described (14). For some assays, 500 ng of purified, recombinant human colipase was added (15). To determine colipase activity, a portion of the extract was heated for 15 min at 65°C to inactivate endogenous lipases. Both lipase and colipase activity were determined using 1 mg of protein from the heat-inactivated extract in the standard pH-stat method (14). All assays were done in duplicate. Another 100 μg of the original extract was analyzed by SDS-PAGE and protein blot as described (16). The anti-PLRP2 and pancreatic lipase-related protein 1 (PLRP1) peptide antibodies were raised in rabbits and purified as previously described (16). Polyclonal antibody against recombinant human pancreatic triglyceride lipase was raised in rabbits. Quantitation of protein bands was done with SigmaGel (SSPS, Chicago, IL). Specific activity was expressed as μmol fatty acid released per min per arbitrary densitometry unit. Protein was measured by the bicinchoninic acid method following the manufacturer's directions (Pierce, Rockford, IL); 5.8 ± 0.48 mg of protein was extracted from each pancreas.

Because of the small extract volume, filtration over an Amicon-100 membrane (Amicon, Beverly, MA) rather than ultracentrifugation was used to remove membrane fragments from

the extract. The supernatant (100 μL) was placed over the membrane and the concentrator was centrifuged at $3000 \times g$ until the sample was nearly dry, $< 10 \mu$L remaining. The membrane was washed by repeatedly pipetting 100 μL of extraction buffer over the membrane. The starting material, the retained fraction and the aqueous fraction or filtrate of the extract were assayed for lipase activity and analyzed by protein blot.

Statistics

Comparisons were done with the software package, SigmaStat (SPSS, Chicago, IL). Pairwise comparisons were done by t test. Comparisons of multiple means were done by one-way ANOVA with Tukey's test. $P < 0.05$ was considered significant and α was 0.05 for both tests. All values are means ± SD.

RESULTS

To determine whether the 4-d-old pancreas contains a colipase-dependent lipase activity, we made extracts of the pancreata from 4-d-old colipase-deficient pups and determined lipase activity. In the absence of added colipase, the extract had an activity of 1450 ± 125 mU/mg protein. The addition of exogenous colipase significantly increased the activity to 3010 ± 243 mU/mg protein ($P < 0.001$ vs. no colipase by t test, $n = 4$). After incubation of the extract with an anti-human pancreatic triglyceride lipase polyclonal antibody that cross-reacts with PLRP2 and another homologue, PLRP1, no activity was detected ($n = 3$) (9).

Similar activities were found in colipase-depleted extracts from the pancreata of 4-d-old wild-type littermates of colipase-deficient pups. The activity in the absence of colipase was 1510 ± 103 mU/mg protein. Adding colipase to the assay increased the activity to 3260 ± 387 mU/mg protein ($P < 0.002$ vs. no colipase by t test, $n = 3$), and preincubation of the extract with the anti-human pancreatic triglyceride lipase polyclonal antibody abolished activity. The wild-type values and the corresponding values from the colipase-deficient pups did not differ.

We next measured lipase activity in colipase-depleted pancreatic extracts from 4-d-old PLRP2-deficient pups. The extract contained 630 ± 50 mU/mg protein of lipase activity ($n = 4$). This activity was significantly decreased compared with the activity in extracts of procolipase-deficient and wild-type mice ($P < 0.001$ for both comparisons by one-way ANOVA with Tukey's test). Adding exogenous colipase to the assay did not increase the activity, which was 620 ± 76 mU/mg protein ($n = 4$). Preincubation with either anti-pancreatic triglyceride lipase antibody or anti-carboxyl ester lipase antibody did not inhibit the activity, 640 ± 63 and 630 ± 71 mU/mg protein, respectively ($n = 3$).

To help identify the source of lipase activity in the extracts from the pancreas of 4-d-old colipase-deficient pups, we took advantage of the tight association of PLRP2 with zymogen granule membranes and separated PLRP2 from soluble lipases (17). The starting extract contained 3300 ± 276 mU/mg protein; 2740 ± 253 mU/mg protein (83%) was retained by the filter, whereas the filtrate contained only 130 ± 18 mU/mg protein (4%) ($n = 3$ for all determinations).

Protein blot analysis with an antibody made against a unique peptide from PLRP2 substantiated this finding (Fig. 1). The antibody, which binds to PLRP2 but does not inhibit activity, recognized a protein band in the extract and in the fraction retained by the filter. A faint band was detected in the sample from the filtrate. No band was present in the extract from the PLRP2-deficient pups, confirming the specificity of the antibody. In contrast, an antibody against a unique PLRP1 peptide recognized a protein in the extract, in the filtrate and in the PLRP2 extract, but not in the fraction retained by the filter as expected for PLRP1, a soluble protein.

We quantitated the amount of PLRP2-reactive protein in the extract and the fraction retained by the filter with scanning densitometry on the protein blot and calculated the specific activity in each sample. The specific activity of the original extract (0.030 ± 0.003) did not differ from that of the retained fraction (0.033 ± 0.003) ($n = 3$).

DISCUSSION

In this study, we demonstrated that PLRP2 accounts for the colipase-dependent activity in pancreatic extracts from 4-d-old mouse pups. Several lines of evidence support this conclusion. An antibody that reacts with PLRP2 as well as with PLRP1 and pancreatic triglyceride lipase completely inhibited the colipase-stimulated triglyceride lipase activity present in pancreatic extracts from 4-d-old colipase-deficient and wild-type mice. Furthermore, colipase-dependent activity was not present in pancreatic extracts from 4-d-old PLRP2-deficient mice, and the same antibody had no effect on the activity in the extract from the PLRP2-deficient pups. Taken together, the analysis of activity in these two extracts suggests that PLRP2 accounts for the colipase-dependent activity in the pancreas of newborn mice.

We further substantiated this conclusion by separating the extracts into two fractions; 95% of the recovered lipase activity was retained by a membrane with a 100,000-Da cutoff, consistent with the tight association of PLRP2 on zymogen granule membranes (17). We demonstrated by immunoblot that the retained fraction contained PLRP2 and did not con tain PLRP1, the only other pancreatic protein in addition to pancreatic triglyceride lipase and PLRP2 known to react with the anti-pancreatic triglyceride lipase antibody that inhibited all activity in the pancreatic extract from 4-d-old colipase-deficient pups. It is important that the calculated specific activities in the starting material and in the retained fraction were identical on the basis of the amount of immunoreactive PLRP2. These findings demonstrate that PLRP2 is predominantly, if not solely, responsible for the triglyceride lipase activity in the pancreata of 4-d-old procolipase-deficient pups. Moreover, the results suggest one potential explanation for the expression of colipase before pancreatic triglyceride lipase in newborns. Colipase is required for maximal activity of PLRP2, an essential lipase for dietary fat digestion in the suckling animal (12).

The finding that colipase stimulates the activity of native mouse PLRP2 strengthens the concept of species-specific differences in the response of various PLRP2 lipases to colipase. To date, the only described native PLRP2 lipases, which were isolated from horses, guinea pigs and coypus, did not have colipase-stimulated activity (18–20). In contrast, the PLRP2 lipases with colipase-dependent activity were all recombinant enzymes (21–23). The colipase-dependent activity in native mouse PLRP2 shows that the species-specific differences in colipase dependence are not a result of analyzing recombinant enzymes.

Our findings also suggest that a different complement of lipases may function in the pancreata of 4-d-old PLRP2-deficient pups than function in wild-type or procolipase- deficient pups. The extracts from the pancreas of the PLRP2-deficient pups contained triglyceride lipase activity. We attempted to identify the source of this activity by inhibition with polyclonal antibodies against pancreatic triglyceride lipase and against carboxyl ester lipase. Neither antibody inhibited the activity, making it unlikely that either of these lipases or PLRP1 account for the lipase activity in the pancreata of 4-d-old PLRP2-deficient pups. The unidentified lipase does not contribute significantly to the activity in the pancreas of the 4-d-old procolipase-deficient or wild-type pups because the antibody against pancreatic triglyceride lipase completely inhibits all activity in these extracts. These findings raise the possibility that the pancreata of 4-d-old PLRP2-deficient pups express a novel lipase or a lipase not normally expressed in the pancreas.

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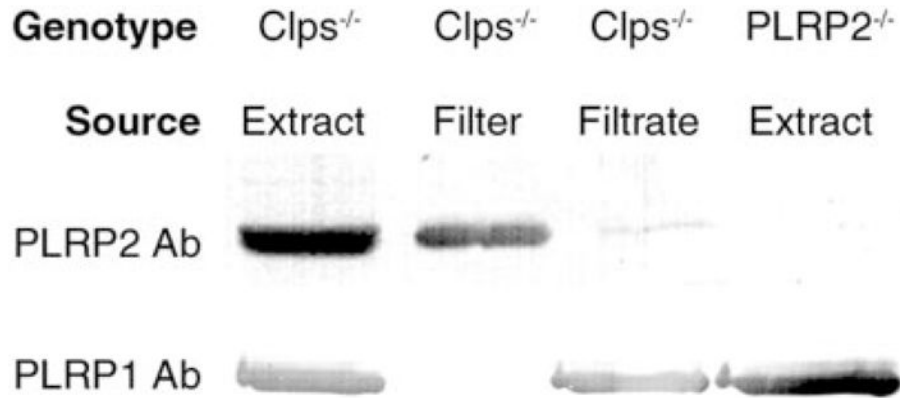
FIGURE 1.
Lipase activity partitions with pancreatic lipase-related protein 2 (PLRP2) extracted from the pancreata of 4-d-old mouse pups. Samples were prepared from extracts made by homogenization, centrifugation and separation of the supernatant over an Amicon-100 filter. The proteins were separated by SDS-PAGE and lipase proteins were detected by protein blot using PLRP2- and PLRP1-specific antibodies. PLRP2 Ab, antibody against a PLRP2-specific peptide; PLRP1 Ab, antibody against a PLRP1-specific peptide.