The β5-Loop and Lid Domain Contribute to the Substrate Specificity of Pancreatic Lipase-related Protein 2 (PNLIPRP2)∗

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Background: Two pancreatic lipases differ in substrate specificities.

Results: Substitution of specific surface loops from one lipase into the other decreases activity against triglycerides and galactolipids.

Conclusion: The β5-loop and lid domain influence lipase substrate specificity.

Significance: Defining the relationship of structure to lipase function is crucial for understanding lipolysis and developing reagents to modulate lipase activity.

Pancreatic triglyceride lipase (PNLIP) is essential for dietary fat digestion in children and adults, whereas a homolog, pancreatic lipase-related protein 2 (PNLIPRP2), is critical in newborns. The two lipases are structurally similar, yet they have different substrate specificities. PNLIP only cleaves neutral fats. PNLIPRP2 cleaves neutral and polar fats. To test the hypothesis that the differences in activity between PNLIP and PNLIPRP2 are governed by surface loops around the active site, we created multiple chimeras of both lipases by exchanging the surface loops singly or in combination. The chimeras were expressed, purified, and tested for activity against various substrates. The structural determinants of PNLIPRP2 galactolipase activity were contained in the N-terminal domain. Of the surface loops tested, the lid domain and the β5-loop influenced activity against triglycerides and galactolipids. Any chimera on PNLIP with the PNLIPRP2 lid domain or β5-loop had decreased triglyceride lipase activity similar to that of PNLIPRP2. The corresponding chimeras of PNLIPRP2 did not increase activity against neutral lipids. Galactolipase activity was abolished by the PNLIP β5-loop and decreased by the PNLIP lid domain. The source of the β9-loop had minimal effect on activity. We conclude that the lid domain and β5-loop contribute to substrate specificity but do not completely account for the differing activities of PNLIP and PNLIPRP2. Other regions in the N-terminal domain must contribute to the galactolipase activity of PNLIPRP2 through direct interactions with the substrate or by altering the conformation of the residues surrounding the hydrophilic cavity in PNLIPRP2.

Before the body can utilize dietary fats, the acyl chains must be cleaved from the parent lipid (1, 2). The digestion and absorption of dietary lipids are highly efficient processes involving several integrated steps, including emulsification, hydrolysis by various lipases, dispersion of the released fatty acids into a protein aqueous environment as mixed micelles with bile salts, and uptake by enterocytes (3). The efficient digestion and absorption of dietary fats and fat-soluble vitamins require the concerted action of multiple lipases with different substrate specificities (4, 5). Hydrolysis starts in the stomach where, in humans, gastric lipase cleaves 15–20% of the fatty acids from triglycerides and continues in the duodenum, where pancreatic lipases complete digestion (6, 7).

Pancreatic triglyceride lipase (PNLIP)2 is the major triglyceride lipase in the duodenum as evidenced by the fat malabsorption seen in patients with isolated PNLIP deficiency (8–11). PNLIP is the archetype of a small lipase subfamily within the α/β-hydrolase fold gene family (12). The family includes PNLIP and two related proteins, PNLIPRP1 and PNLIPRP2. The lipases share 70% amino acid identity and have super-imposable α-carbon backbones (Fig. 1A) (13–15). Each lipase has two domains, an N-terminal domain from residues 18 to 353 and a C-terminal domain from residues 354 to 466. The N-terminal domain consists of an α/β-hydrolase fold, which is present in other lipases and esterases (16). This domain also contains the Ser-His-Asp catalytic triad defined by analogy to serine proteases and confirmed by site-directed mutagenesis (15, 17, 18). A surface loop, the lid domain, covers the active site of PNLIP. In the presence of mixed micelles or non-ionic detergents, a 29-Å hinge movement of the PNLIP lid domain and a smaller movement of the β5-loop open and configure the active site (Fig. 1, A and B) (19–23). In contrast, the lid domain of PNLIPRP2 is more mobile and can adopt an open conformation in the absence of amphiphiles (24). The C-terminal domain of each lipase has a β-sandwich structure similar to the C2 domain of other lipid-binding proteins such as 15-lipoxygenase, Clostridium perfringens α-toxin, phospholipase A2, and synaptotagmin I (25).

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Despite their marked structural homology, these three homologs differ in their enzymatic properties (12). PNLIP cleaves acyl chains from triglycerides (26, 27). Even though PNLIP can bind phosphatidylcholine in the active site, it has no significant activity against phospholipids or galactolipids (19, 27, 28). In contrast, PNLIPRP2 cleaves acyl chains from triglycerides, galactolipids, and phospholipids (14, 29, 30). PNLIP has an absolute requirement for colipase in the presence of bile salt micelles. PNLIPRP2 does not, although its activity is stimulated by colipase (31–33). PNLIPRP1 has no defined lipase activity (13, 34, 35). The molecular mechanism for the differences in substrate specificity must be determined by specific structural domains in the individual lipases.

Even with tremendous progress in understanding lipase function, the molecular details underlying the binding of a substrate molecule in the active site are limited. Much of our knowledge about pancreatic lipase substrate specificity comes from predictions based on the structures of human PNLIP crystallized with substrate analogs. The nucleophilic Ser-160 of PNLIP, Glu-91 and Asp-92 occupy different positions in PNLIPRP2 compared with the corresponding residues in PNLIP, Glu-90 and Glu-91. In particular, the position of the Cα carbon atoms of Asp-92 in PNLIPRP2 and Glu-91 in PNLIP differs by 4.7 Å. Consequently, the hydrogen bond observed in PNLIP between Glu-91 and Trp-260 in the open lid does not exist in PNLIPRP2. The conformation of Glu-91 and Asp-92 in PNLIPRP2 could influence the binding of the polar headgroup of phospholipids or galactolipids in the active site of PNLIPRP2. In addition, the conformation of the β9-loop shows differing conformations of several side chains crucial to acyl chain binding. Two hydrophobic residues from the PNLIP β9-loop, Leu-231 and Phe-233, interact with the alkyl chain of a phosphate inhibitor and likely stabilize the acyl-enzyme intermediate formed during lipolysis (36). These residues are conserved in all pancreatic lipases, but whereas Phe-233 of PNLIP and Phe-234 of PNLIPRP2 superimpose well, the Cα carbons of Leu-232 in PNLIPRP2 and Leu-231 in PNLIP are 3.4 Å apart. These findings suggest the hypothesis that the β5- and β9-loops and the lid domain contribute to the differences in substrate specificity between PNLIP and PNLIPRP2.

In this study, we tested this hypothesis by creating chimeric proteins by exchanging the respective β5- and β9-loops and the lid domain between human PNLIP and PNLIPRP2. We expressed and purified the chimeras and determined their activity against short-, medium-, and long-chain triglycerides and against a galactolipid, digalactosyldiacylglycerol. We found that the lid domain and the β5-loop influence substrate specificity significantly.

**Experimental Procedures**

**DNA Manipulations**—The cDNAs encoding for mature human PNLIP and PNLIPRP2 and for PNLIP/PNLIPRP2 and PNLIPRP2/PNLIP chimeras were amplified by regular and overlap PCR, respectively. The amplified cDNAs were subcloned into the yeast protein expression vector pHILSI, in which lipase native secretion signal peptide was replaced by the yeast PHO1 secretion peptide (Invitrogen). All other domain swap mutations were introduced into the parent cDNA in the pHILSI vector using XL QuikChange site-directed mutagen-
sis kit (Stratagene). All of the DNA constructs were verified by dideoxynucleotide sequencing.

Recombinant Protein Expression, Production, and Purification—All recombinant lipase proteins were produced in Pichia pastoris yeast strain GS 115 following the manufacturer’s manual (Invitrogen). Each plasmid DNA was linearized by BglII and purified by phenol chloroform method. The competent yeast cells were transformed with purified DNAs by electroporation, and the resultant yeast transformants were screened by lipase activity assay and/or immunoblot analysis of culture medium after 24 h of methanol induction as described previously (31, 37). All proteins were robustly secreted indicating that the chimeras were not misfolded.

One highly expressing colony for each of the recombinant lipases was then used to produce a large quantity of recombinant protein (31, 37). After 24–48 h of methanol induction, cell-free culture medium was clarified by filtration and concentrated to ~50 ml over a Pellicon XL Biomax 10 membrane (Millipore). The concentrated protein sample was then dialyzed at 4 °C overnight against distilled H2O containing 2 mM benzamidine. Each recombinant lipase protein was purified to homogeneity by one-step chromatography using a Mono S FPLC column (GE Healthcare) according to the protocols described previously (31, 37). Fractions of purified lipase were evaluated and analyzed by lipase activity assay and 10% SDS-polyacrylamide gel staining using GelCode Blue Stain Reagent (Pierce). The pooled purified recombinant protein was then concentrated and buffer-exchanged to 25 mM Tris-HCl, pH 8.0. Protein concentration was determined by spectrophotometry at 280 nm. The extinction coefficient of each lipase was calculated using ProtParam program at EXPASY. The final yield ranged from 10 to 40 mg/liter for all of the purified proteins. The homogeneity and integrity of each purified lipase protein was verified by SDS-polyacrylamide gel staining.

Standard Lipase Activity Measurements—The activity of lipases was determined in bulk by measuring the release of fatty acids from mechanically stirred emulsions of tributyrin, trioctanoin, or triolein as described previously (38, 39). Unless otherwise stated, the assay was conducted with or without 5 M octanoin, or triolein as described previously (38, 39). Unless otherwise stated, the assay was conducted with or without 5 M octanoin, or triolein as described previously (38, 39). Unless otherwise stated, the assay was conducted with or without 5 M octanoin, or triolein as described previously (38, 39). Unless otherwise stated, the assay was conducted with or without 5 M octanoin, or triolein as described previously (38, 39). Unless otherwise stated, the assay was conducted with or without 5 M octanoin, or triolein as described previously (38, 39).

The activity of all four lipases was determined in the pH-stat in the presence of 4 mM NaTDC and a 5 M excess of colipase. Each assay contained 2.6 mM lipase. The activity was determined from the slope of the titration curve. All results are expressed relative to the activity of PNLIP against tributyrin (4-carbon acyl chain) (5063 ± 8.5 units/mg protein). Trioctanoin has an 8-carbon acyl chain; triolein has an 18-carbon acyl chain. The values are the mean ± S.D. of three separate measurements. White bars, PNLIP; black bars, PNLIPRP2; spaced hatched bars, PNLIP/PNLIPRP2; thinly spaced hatched bars, PNLIPRP2/PNLIP. Pairwise comparison of PNLIP activity with the other activities is significantly different for all comparisons (p < 0.001). Pairwise comparisons of PNLIPRP2, PNLIP/PNLIPRP2, and PNLIPRP2/PNLIP were not significantly different.

Results

Construction of N- and C-terminal Chimeras—Because PNLIP and PNLIPRP2 have distinct N- and C-terminal domains, we constructed chimeras of PNLIP and PNLIPRP2 to determine whether one or both of these domains contributes to substrate specificity. One chimera contained the PNLIP N-terminal domain and the PNLIPRP2 C-terminal domain (PNLIP/PNLIPRP2), and the other chimera included the PNLIPRP2 N-terminal domain and the PNLIP C-terminal domain (PNLIPRP2/PNLIP). Both were expressed in P. pastoris and purified in a single step. We then tested activity against triglycerides with varying acyl chain lengths and against digalactosyldiacylglycerol in various concentrations of NaTDC with and without colipase.

Triglyceride Lipase Activity—First, we tested activity against tributyrin, trioctanoin, and triolein emulsified in 4 mM NaTDC in the presence of a 5-fold molar excess of colipase (Fig. 2). As expected, the activity of PNLIP decreased with increasing acyl chain length, and the activity of PNLIP was significantly higher than the activity of PNLIPRP2 for all substrates. Of note, both of the chimeras had activities that were comparable with the activity of PNLIPRP2 for each of the substrates. These results suggest that the structures of the PNLIPRP2 N- and C-terminal domain influence activity more than the corresponding domains from PNLIP.

To better understand the effects of the PNLIPRP2 domains on the activity of the chimeric lipases, we determined the activity of all four lipases against tributyrin, trioctanoin, and triolein in various concentrations of NaTDC with and without a 5 M excess of colipase (Fig. 3). As reported before, PNLIPRP2 had lower activity against tributyrin than PNLIP, and the activity of
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- PNLIP
- PNLIPRP2
- PNLIP / PNLIPRP2
- PNLIPRP2 / PNLIP
PNLIPRP2 was not stimulated by colipase as was the case with PNLIP (Fig. 3, left-hand panels). The activity of the PNLIP/PNLIPRP2 chimera was intermediate between the activity of PNLIP and PNLIPRP2 (Fig. 3). Like PNLIP, the PNLIP/PNLIPRP2 chimera was completely inhibited at 4 mM NaTDC. Similarly, colipase stimulated the activity of the PNLIP/PNLIPRP2 chimera but did not restore the activity in 4 mM NaTDC to the level of activity in 0.5 mM NaTDC as it did with PNLIP. The PNLIP/PNLIPRP2 chimera had an activity curve and colipase response similar to that of PNLIPRP2 (Fig. 3). In the absence of colipase, increasing NaTDC concentrations did not completely inhibit the PNLIPRP2/PNLIP chimera similar to the results with PNLIPRP2.

With trioctanoin, the PNLIP/PNLIPRP2 chimera had about 2-fold higher activity than PNLIPRP2, but the activity was 9-fold lower than PNLIP (Fig. 3, middle panels). PNLIPRP2 and PNLIP/PNLIPRP2 had similar activity (Fig. 3, middle panels). Increasing concentrations of NaTDC inhibited all four lipases, and colipase restored activity. As observed in the tributyrin assays, colipase increased PNLIP activity in 4 mM NaTDC to the level measured in 0.5 mM NaTDC. Colipase-stimulated activity of PNLIPRP2, PNLIP/PNLIPRP2, and PNLIPRP2/PNLIP did not reach the levels measured in 0.5 mM NaTDC.

With triolein, the activity of the two chimeras was similar to the activity of PNLIPRP2 (Fig. 3, right-hand panels). The activity of PNLIP was about 10-fold higher than the activity of the other lipases. For all lipases, micellar concentrations of TDC inhibited activity, and colipase restored the activity to the level measured below the critical micellar concentration for TDC (1.9 mM). As reported previously, PNLIPRP2 required oleic acid to overcome a long lag time (31). Neither of the chimeras had an appreciable lag time, and oleic acid was not required for full activity.

**Colipase Activation of the Chimeras**—Because colipase appeared to activate the two chimeras to a lesser extent than it activated PNLIP with tributyrin and trioctanoin, we next measured the effect of colipase over a range of colipase concentrations in the presence of a large excess of trioctanoin emulsified in 4.0 mM NaTDC and a constant concentration of each lipase (Fig. 4). The activity of each lipase saturated with increasing concentrations of colipase suggested a specific interaction of colipase with each lipase and the substrate. We then used nonlinear regression to determine the concentration of colipase that restored half-maximal activity to each lipase (apparent $K_a$).

Given the large excess of substrate in the assays, the apparent $K_a$ value likely reflects the interaction between colipase and lipase rather than the interaction of colipase and the substrate emulsion, although the interaction probably occurs at the substrate interface. The apparent $K_a$ value for PNLIPRP2 and the two chimeras was 3–5-fold higher than the apparent $K_a$ value for PNLIP, indicating that the chimeras have a lower affinity for colipase (Table 1).

**Galactolipase Activity of the Chimeras**—We then determined the activity of the lipases against a galactolipid. We performed the bulk phase assays using a pH-stat method and varying amounts of NaTDC in the presence of colipase. Neither PNLIP nor PNLIP/PNLIPRP2 had detectable galactolipase activity at any NaTDC concentration (Fig. 5). In contrast, PNLIPRP2 and PNLIPRP2/PNLIP had identical activity at all bile salt concentrations tested. The activity of both was stimulated by bile salts below the critical micellar concentration of NaTDC. At 4 mM NaTDC, removing colipase or including 5–10-fold molar excess colipase in the reaction mixture had no effect on the activity of PNLIPRP2 or PNLIPRP2/PNLIP against galactolipids (data not shown). The results clearly show that the
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**Table 1**

| Lipase                  | Apparent $K_{App}$ (nM) |
|-------------------------|--------------------------|
| PNLIP                   | 3.9 ± 0.66               |
| PNLIPRP2                | 14.6 ± 2.79              |
| PNLIP/PNLIPRP2          | 19.0 ± 1.32              |
| PNLIPRP2/PNLIP          | 12.4 ± 0.68              |

Data from three separate experiments were fit by nonlinear regression with a rectangular hyperbolic function. The $K_{App}$ is reported as nM ± S.D.

![Graph](image)

**Figure 5.** Galactolipase activity of PNLIP, PNLIPRP2, PNLIP/PNLIPRP2, and PNLIPRP2/PNLIP. Activity was measured in the pH-stat in the presence of 4 mM NaTDC. Each assay contained 5 mg of digalactosyldiacylglyceride, 2.6 nM each lipase, and 5-fold molar excess colipase. Black circles, PNLIPRP2; open circles, PNLIPRP2/PNLIP; black triangles, PNLIP; open triangles, PNLIP/PNLIPRP2. Each point represents a single assay.

structural determinants for galactolipase activity reside in the N-terminal domain of PNLI PRP2.

**Exchange of the Lid Domain and the β5- and β9-Loops**—To further define the molecular structures responsible for the galactolipase activity of PNLI RP2 lipases, we performed switches of smaller domains between PNLI and PNLI RP2. We focused on the surface loops (lid domain, the β5-loop, and the β9-loop) surrounding the active site (Fig. 1). First, we substituted the regions in PNLI with the corresponding sequence from PNLI RP2 to create a chimera of PNLI with each of the PNLI RP2 domains singly or in combination (Fig. 6). Each construct was expressed in *P. pastoris* and purified as described under “Experimental Procedures.”

**Triglyceride Lipase Activity of the Lid Domain and the β5- and β9-Chimeras**—Before testing galactolipase activity, we determined the effect of the domain switches on the activity against triglycerides in the presence of 4 mM NaTDC and excess colipase (Fig. 7). All of the chimeras with substitution of the PNLI RP2 surface loops into PNLI significantly decreased activity against all three triglycerides. Substitution of the PNLI RP2 β5-loop or lid domain into PNLI had a larger effect on activity than substituting the β9-loop ($p < 0.001$). The largest effect on activity occurred when the PNLI RP2 lid domain was substituted into PNLI. The presence of the PNLI RP2 lid domain decreased activity against all three triglycerides to the level of activity measured for PNLI RP2. Including the β5-loop and β9-loop singly or together with the lid domain made little difference in activity compared with the lid domain substitution alone.

**Galactolipase Activity of the Lid Domain and the β5- and β9-Chimeras of PNLI RP2**—We then took another approach and determined whether substitution of the PNLI RP2 lid domain, β5-loop, or β9-loop into PNLI RP2 affected galactolipase and neutral lipase activity compared with the activity of PNLI RP2 (Table 3). Exchange of the PNLI RP2 β5-loop with the β5-loop from PNLI impaired galactolipase activity completely. The chimera also had significantly decreased activity against tributyrin and trioctanoin. Unexpectedly, exchange of the β9-loop from PNLI increased activity against galactolipids about 1.6-fold but had no effect on activity against the neutral lipids. Substitution of the PNLI RP2 lid domain with the analogous domain from PNLI decreased activity against galactolipids about 10-fold. In contrast, the lid domain chimera had preserved activity against tributyrin and higher activity against trioctanoin. Most of the effect resided in the 3′-half of the lid domain because PNLI RP2/PNLILid3′ galactolipase activity was decreased about 5-fold, and the activity against tributyrin and trioctanoin was decreased 1.5- and 2-fold, respectively. In contrast, substitution of the 5′-half of the PNLI lid into PNLI RP2 (PNLI RP2/PNLILid5′) increased activity against both the galactolipid and neutral lipids.

**Discussion**

In this study, we utilized domain swaps between PNLI and PNLI RP2 to provide additional and novel insight into the molecular mechanisms for the differences in substrate specificity between PNLI and PNLI RP2 (12). First, the differences in activity against neutral lipids are dominated by the properties of the PNLI RP2 N- or C-terminal domains. The decreased activity of the N- and C-terminal chimeras is largely explained by impaired interactions with colipase and by structural differences in the lid domain and the β5-loop. Second, the structural determinants of galactolipase activity reside solely in the N-terminal domain of PNLI RP2. The lid domain and the β5-loop in the N-terminal domain of PNLI RP2 strongly influence galactolipase activity.

Regardless of whether the N- or C-terminal domain originated from PNLI RP2, the triglyceride lipase activity of the chimeras more closely resembled that of PNLI RP2 than that of PNLI. This finding agrees with the report of similar domain chimeras for the horse lipases (41). The presence of the horse PNLI RP2 C- or N-terminal domain in a chimera with the opposite PNLI domain resulted in activity against tributyrin that was similar to PNLI RP2 and 20-fold lower than horse PNLI. Similarly, a chimera between the N-terminal domain of guinea pig PNLI RP2 and the C-terminal domain of human PNLI had a specific activity close to the activity of the native guinea pig PNLI RP2 (42).
The decreased activity of the human domain chimeras is partly explained by impaired interactions with colipase. Both PNLIP/PNLIPRP2 and PNLIPRP2/PNLIP have an apparent $K_d$ value for colipase that is similar to the value for PNLIPRP2 and about 3–5-fold higher than the value for PNLIP. Thus, both the N- and C-terminal domains contribute to the interaction of the lipases with colipase. Our results are similar to the data reported in a previous publication (41) on the functional properties of domain chimeras between horse PNLIP and horse PNLIPRP2. Colipase did not stimulate the activity of either chimera suggesting the interaction with colipase was impaired by the domains derived from PNLIPRP2.

Because binding of colipase to PNLIP is mediated through a pincer mechanism by residues in the C-terminal domain and
we investigated the contributions of the affinity for the PNLIP/PNLIPRP2. With some or all four differing residues might decrease colipase for colipase, the result suggests that the loss of the interactions explain the decreased affinity of the PNLIP/PNLIPRP2 chimera to the corresponding amino acid in PNLIPRP2, Y421N, (20, 44). Of these four, only Tyr-421 has been tested for its C-terminal domain could decrease affinity of colipase for interactions with colipase. Of the PNLIPRP2 N-terminal domain could result in weaker interaction of the PNLIPRP2 lid domain to activity against neutral lipids (46). We first served in the PNLIPRP2 lid domain, the increased mobility of critical lid domain residues (Asn-258 and Val-264) are con-

The values are ± 1 S.D. ND = none detected.

| Lipase          | Activity | Substrate |
|-----------------|----------|-----------|
| PNLIPRP2        | 110 ± 7.0* | 203 ± 9.0 | 191 ± 6.0 |
| PNLIP           | ND       | 45 ± 3.0***| 29 ± 5.0***|
| PNLIP/PNLIPRP2b5| 154 ± 9.0 | 242 ± 5.0** | 190 ± 8.0 |
| PNLIPRP2/PNLIP  | 12 ± 5.0**| 184 ± 2.0 | 315 ± 6.0***|
| PNLIPRP2/PNLIPLid45 | 190 ± 21**| 355 ± 13***| 498 ± 11***|
| PNLIPRP2/PNLIPLid3* | 26 ± 7.0***| 130 ± 5.0***| 100 ± 3.0***|

* p = 0.011 by Student’s t test.

### Table 3

Lipase activity of PNLIPRP2 chimeras with PNLIP surface loops

All assays were done by the pH-stat method with digalactosyldiacylglyceride (DGDG) as described under “Experimental Procedures.” Activity was in units/mg. The values are ± 1 S.D. ND = none detected. Statistical analysis was done by one-way analysis of variance with a Bonferroni test method for multiple compar-

The steric hindrance of these residues would prevent produc-

The large effect of the human PNLIPRP2 lid domain on the neutral lipase activity of human PNLIP mirrors that reported for lid domain exchanges between the homologous rat lipases (39). The explanation for the lid domain influence on activity likely rests on the observation that the PNLIPRP2 lid is more mobile in the open position (14, 24). Because the open PNLIP lid domain contributes to one acyl chain-binding site, any change in the confirmation of the lid domain can potentially alter the binding affinity of the mutant lipase for substrate (36, 46).

In contrast, exchange of the β5-loop, β9-loop, and the lid domain in PNLIPRP2 with the corresponding domains from PNLIP had little effect on the activity of the chimeras against tributyrin or trioctanoin. The largest effect was a 4–6-fold decrease in activity when the PNLIPRP2 β5-loop was replaced with the PNLIP loop. Exchanges involving the β9-loop or lid domain had smaller effects on activity.

As opposed to the effects on activity against neutral lipids, activity against galactolipids was totally conferred by the PNLIPRP2 N-terminal domain, and colipase had no effect on activity. Likewise, the phospholipase activity of guinea pig PNLIPRP2 is determined by structures in the N-terminal domain (42). The molecular structure around the active site of PNLIPRP2 lipases must differ from the structure of PNLIP in a way that allows accommodation of the polar headgroup of galactolipids and phospholipids.

By analyzing chimeras of the β5-loop, β9-loop, and lid domain from one lipase on the backbone of the other lipase, we were able to show that both the 3’-half of the lid domain and β5-loop contribute to the galactolipase activity of PNLIPRP2. Our finding that the β5-loop is crucial for galactolipase activity is similar to a report demonstrating that the β5-loop influences substrate specificity in extracellular phospholipase A1 (47).

Molecular modeling of digalactosyldiglyceride suggests the digalactose polar headgroup can fit into a cavity in the active site of PNLIPRP2 (46). In lipases lacking activity against polar lipids, the cavity does not form. In particular, human PNLIP does not have a cavity for the polar headgroup because the region is occupied by Asp-265 and Arg-274 of the lid domain. The steric hindrance of these residues would prevent productive binding of polar lipids into the active site of human PNLIP.

Rather than hinder binding of polar lipids, the properties of the β5-loop may increase the hydrophilicity of the active site and enhance binding of polar lipids. It was noted that the water-accessible area of the β5-loop in guinea pig PNLIPRP2 is more hydrophilic than the β5-loop in PNLIP, and it was suggested that this property contributes to the hydrophilic cavity where the polar headgroup of digalactosyldiglyceride is predicted to reside (28, 46). To test this hypothesis, we calculated the water-
accessible area of the human PNLIPRP2 β5-loop. The hydrophilic/hydrophobic balance of the exposed residues was slightly higher (2.3) than that measured in human PNLIP (1.4) (28). The value is lower than that measured for the β5-loop of guinea pig PNLIPRP2 (5.4) and may not provide an adequate explanation for the effect of replacing the β5-loop in human PNLIPRP2 with the corresponding loop from human PNLIP (28).

Another possibility is suggested by a polar interaction that forms between Glu-101 in the β3-loop and Trp-269 in the open lid domain of human PNLIP (36). The corresponding residue in PNLIPRP2, Glu-102, assumes a different orientation, and the interaction with Trp-269 may not form (24). If Glu-102 assumes the same position in PNLIPRP2/PNLIPβ5 as the position of Glu-101 in PNLIP, the interaction with Trp-269 may form and bring other lid domain residues into the hydrophilic crevice as occurs in the open conformation of PNLIP. The resolution of the different possibilities would be aided by a crystal structure of the PNLIPRP2/PNLIPβ5 chimera.

In summary, our study suggests that the differences in activity against neutral lipids between PNLIP and PNLIPRP2 are influenced by the alterations in the interaction of collapse with each lipase and by the structure of the lid domain and β5-loop. These same structures mediate the ability of PNLIPRP2 to hydrolyze galactolipids. However, the lid domain and β5-loop do not completely account for the activity. Other regions in the N-terminal domain must contribute to the galactolipid activity of PNLIPRP2 by direct interactions with the substrate or by altering the conformation of the residues surrounding the hydrophilic cavity in PNLIPRP2.

Author Contributions—X. X. constructed, expressed, purified, and assayed all of the recombinant proteins described in this study. He conceived the project, designed the chimera, contributed to the writing of the manuscript. M. E. L. directed all aspects of this work. He conceived the project, designed the chimera, and contribution to lipolysis of gastric and pancreatic lipases during a test meal in humans. Gastroenterology 105, 876–888

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