To specify and localize carboxyl-terminal domain functions of human hepatic lipase (HL) and human lipoprotein lipase (LPL), two subdomain chimeras were created in which portions of the carboxyl-terminal domain were exchanged between the two lipases. The first chimera (HL-LPLC1) was composed of residues 1–344 of human HL, residues 331–388 of human LPL, and residues 415–476 of human HL. The second chimera (HL-LPLC2) consisted of just two segments, residues 1–414 of human HL and residues 389–448 of human LPL. These chimeric constructs effectively divided the HL C-terminal domain into halves, with corresponding LPL sequences either in the first or second portion of that domain. Both chimeras were lipolytically active and hydrolyzed triolein emulsions to a similar extent compared with native HL and LPL. Heparin-Sepharose chromatography demonstrated that HL-LPLC1 and HL-LPLC2 eluted at 0.80 and 1.3 M NaCl, respectively, elution positions that corresponded to native HL and LPL.

Hence, substitution of LPL sequences into the HL carboxyl-terminal domain resulted in the production of functional lipases, but with distinct heparin binding properties. In addition, HL-LPLC2 trioleinase activity was responsive to apoC-II activation, although the fold stimulation was less than that observed with native LPL. Moreover, an apoC-II fragment (residues 44–79) was specifically cross-linked to LPL and HL-LPLC2, but not to HL or HL-LPLC1. Finally, both chimeras hydrolyzed phospholipid with a specific activity similar to that of HL, which was unaffected by the presence of apoC-II. These findings indicated that in addition to a region found within the amino-terminal domain of LPL, apoC-II also interacted with the last half of the carboxyl-terminal domain (residues 389–448) to achieve maximal lipolytic activation. In addition, the relative heparin affinity of HL and LPL was determined by the final 60 carboxyl-terminal residues of each enzyme.

Hepatic lipase (HL) and lipoprotein lipase (LPL) are members of a lipase gene family that also includes pancreatic lipase (1–3). X-ray crystallography of pancreatic lipase crystals shows the enzyme to be composed of two domains, with a larger amino-terminal domain containing the catalytic site joined by a short spanning region to a smaller carboxyl-terminal domain (4). Based on amino acid sequence homology, the conservation of disulfide bridges, and similarity of lipolytic function, HL and LPL are proposed to have a similar overall three-dimensional structure to pancreatic lipase (5). Multiple functional characteristics of HL and LPL have been identified, including the catalytic site, surface loop region, heparin affinity, and lipid and receptor binding properties as well as the requirement of two subunits for lipolytic activity. However, although these enzymes share a number of structural and functional similarities, specific differences in substrate specificity, cofactor requirements, and relative heparin affinity distinguish each enzyme. The creation and analysis of chimeric enzymes have been used to exploit these differences to localize various functions of these lipases to specific regions within the enzyme.

Initially, a chimeric lipase was constructed that consisted of the amino-terminal 329 amino acids of rat HL joined to the carboxyl-terminal 136 amino acids of human LPL (6). Based on the pancreatic lipase crystal structure, this division in HL and LPL separated the two putative domains of the enzymes. This HL chimera hydrolyzed lipid- and water-soluble substrates with catalytic constants similar to those of native rat HL, suggesting that the HL amino-terminal domain was responsible for the catalytic character of the chimeric molecule. However, a monoclonal antibody, whose epitope was mapped to the carboxyl-terminal domain of LPL, eliminated lipolytic activity, but had no effect on the esterolytic properties of the HL chimer (6). Similar results were reported when a monospecific anti-HL polyclonal antibody specific for the carboxyl-terminal domain of HL was reacted with a chimeric enzyme composed of the amino-terminal domain of human LPL joined to the carboxyl-terminal domain of human HL (LPL chimera) (7). Consistent with these findings was the observation that a bovine LPL proteolytically truncated at residue 390 was unable to bind to chylomicrons (8). These results provided evidence that the carboxyl-terminal domains of HL and LPL participate in lipolysis and contain regions responsible for lipid binding. More recently, it was shown for human LPL that substitution of alanine for tryptophan at position 390, 393, or 394 resulted in a significant reduction in catalytic activity against long-chain lipids.
triacylglycerols, providing additional support for a crucial role of the carboxy-terminal domain in lipolysis (9).

Properties associated with native LPL such as activation by apoC-II and sensitivity to high salt (1 M NaCl) were also observed for LPL chimeras (7, 10), suggesting that the amino-terminal domain of LPL was responsible for these characteristics. In contrast, localization of HL and LPL heparin-binding regions has been more contentious; a variety of studies have concluded that elements in either the amino-terminal (11) or carboxy-terminal (10) domain or in both domains (7, 12) are responsible for heparin affinity. More recently, analysis of fragments of the LPL C-terminal domain expressed in Escherichia coli indicated that amino acids within residues 404–430 contain a heparin-binding site (13).

To supplement structure-function information provided by previously reported chimeras of HL and LPL that divided the monomer molecule between domains, this report describes chimeras that subdivide the carboxy-terminal domain to specifically examine the role of these subdomain regions in lipase activity, heparin binding, and cofactor activation. These new chimeric enzymes were lipolytically active, but differed markedly in their response to apoC-II activation and their relative heparin affinities and suggests that this same putative region of LPL participates in apoC-II activation.

EXPERIMENTAL PROCEDURES

Chimera Construction—Human HL and LPL cDNA fragments were joined together by creating overlapping PCR fragments allowing the extension and amplification of full-length chimeric cDNAs. A schematic representation of the amino acid sequence of each chimera as well as the parental lipases are shown in Fig. 1. For the HL-LPLC1 chimera, 70 residues of human HL (residues 345–414) were replaced with the analogous region in human LPL (residues 331–388), a 58-residue segment that is 12 amino acids shorter in length. This change resulted in the loss of an N-glycosylation site at position 374 of human HL and the addition of a potential N-glycosylation site at position 359 from human LPL. Thus, the newly created chimera (HL-LPLC1) has the same total number and origin of four potential N-glycosylation sites as native HL, three contributed by HL at positions 19, 55, and 339 and one by LPL at position 359. By contrast, for the HL-LPLC2 chimera, residues 415–476 of HL were replaced with residues 389–448 of LPL, a segment that is only two amino acids shorter in length. In this instance, the number and origin of potential N-glycosylation sites were not altered in comparison with the human HL sequence.

The HL-LPLC1 chimera consisted of three segments: residues 1–344 of the human HL amino terminus, residues 331–388 of the human LPL carboxy-terminal domain, and residues 415–476 of the human HL carboxy-terminal. The HL-LPLC2 chimera consisted of two segments: residues 1–414 of human HL and residues 389–448 composing the 60 carboxy-terminal amino acids of human LPL.

Primers and PCR Amplification—For primers defining the 5’ and 3’ termini of the construct, restriction endonuclease sites were added to allow directional cloning. The LPL portion of the HL-LPLC1 chimera was amplified using splicing (HLLPL335FOR, ACT GAT TCA TTC TCG GCC ACC AGC ACA GGG CCG), the reverse primer encoded residues 415–420 of HL and containing upstream sequences derived from codons 385–390 of LPL (LPLHL415FOR, ACT GAT TCA TTC TCG GCC ACC AGC ACA GGG CCG). The reverse primer consisted of a 4-base cap, a BamHI restriction site, and the reverse complement to 21 nucleotides found within the 3’-untranslated region of the HL cDNA (HIND5PKHHL, ACG TAA GCT TGG CAC CAT GGA CAC AAG TCC CTT GGT T). The reverse primer encoded residues 331–344 of HL and contained upstream splicing sequences derived from codons 331–335 of LPL. Amplification of full-length chimeric cDNAs was achieved by using HIND5PKHHL as a forward primer and a reverse primer encoding sequences derived from codons 410–414 of HL and upstream sequences derived from residues 389–394 of LPL. The amplified PCR product was combined with the third PCR product to produce a full-length chimeric cDNA using HIND5PKHHL and Bam3PPL as flanking primers in the fifth and final PCR.

The amino-terminal portion of the HL-LPLC2 chimera was amplified using HIND5PKHHL as a forward primer and a reverse primer encoding residues 409–414 of HL and containing upstream splicing sequences derived from codons 331–335 of LPL. Amplification of full-length chimeric cDNAs was achieved by using HIND5PKHHL as a forward primer and a reverse primer encoding sequences corresponding to residues 389–394 of HL and upstream sequences derived from codons 410–414 of HL (HLLPL388FOR, GTC CAC ATC ATC ATC AGG TCA GAC GAT GGT CTA GGT). The reverse primer consisted of a 4-base cap, a BamHI restriction site, and the reverse complement to 21 nucleotides found within the 3’-untranslated region of the LPL cDNA (BAM3PLPL, ACG TGG ATC CAA GGA CAG AAG TCC CTT GGT TGT GTG T). Following purification, the two PCR products were combined together in a third PCR along with the flanking primers HLLPL331FOR and Bam3PPL to generate a full-length chimera. The amino-terminal portion of the HL-LPLC2 chimera was amplified using HIND5PKHHL as a forward primer and a reverse primer encoding residues 409–414 of HL and containing upstream splicing sequences derived from codons 331–335 of LPL. Amplification of full-length chimeric cDNAs was achieved by using HIND5PKHHL and Bam3PPL as flanking primers in the fifth and final PCR.

DNA Transfection and Expression—Full-length cDNAs were purified, digested, and inserted into the pcDNA3 expression vector (Invitrogen). The amplified cDNA was used as a template with a forward primer encoding residues 410–414 of HL and upstream sequences derived from codons 389–394 of LPL, and downstream sequences corresponding to residues 331–335 of LPL (LPLHL415FOR, ACT GAT TCA TTC TCG GCC ACC AGC ACA GGG CCG). The reverse primer encoded sequences derived from codons 410–414 of HL and upstream sequences derived from residues 389–394 of LPL. The amplified PCR product was combined with the third PCR product to produce a full-length chimeric cDNA using HIND5PKHHL and Bam3PPL as flanking primers in the fifth and final PCR.

The amino-terminal portion of the HL-LPLC2 chimera was amplified using HIND5PKHHL as a forward primer and a reverse primer encoding residues 409–414 of HL and containing upstream splicing sequences derived from codons 331–335 of LPL. Amplification of full-length chimeric cDNAs was achieved by using HIND5PKHHL as a forward primer and a reverse primer encoding sequences corresponding to residues 389–394 of HL and upstream sequences derived from codons 410–414 of HL (HLLPL388FOR, GTC CAC ATC ATC ATC AGG TCA GAC GAT GGT CTA GGT). The reverse primer consisted of a 4-base cap, a BamHI restriction site, and the reverse complement to 21 nucleotides found within the 3’-untranslated region of the LPL cDNA (BAM3PLPL, ACG TGG ATC CAA GGA CAG AAG TCC CTT GGT TGT GTG T). Following purification, the two PCR products were combined together in a third PCR to form the full-length chimeric cDNA using the flanking primers HIND5PKHHL and Bam3PPL.

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DNA Transfection and Expression—Full-length cDNAs were purified, digested, and inserted into the pcDNA3 expression vector (Invitrogen). The amplified cDNA was used as a template with a forward primer encoding residues 410–414 of HL and upstream sequences derived from codons 389–394 of LPL, and downstream sequences corresponding to residues 331–335 of LPL (LPLHL415FOR, ACT GAT TCA TTC TCG GCC ACC AGC ACA GGG CCG). The reverse primer encoded sequences derived from codons 410–414 of HL and upstream sequences derived from residues 389–394 of LPL. The amplified PCR product was combined with the third PCR product to produce a full-length chimeric cDNA using HIND5PKHHL and Bam3PPL as flanking primers in the fifth and final PCR.
through a 2-ml hydroxylapatite column to remove contaminating proteins and then diluted with an equal volume of 20% glycerol and 5 mM barbital, pH 7.2, before it was loaded onto a second heparin-Sepharose column (1 × 5 cm). The column was washed with 50 ml of 0.5 M NaCl and 5 mM barbital, pH 7.2, and then it was eluted with 50 ml of 1 M NaCl (or 1 M NaCl for HL-LPLC2 and LPL) and 5 mM barbital, pH 7.2. The collected eluant was concentrated in an Amicon filtration unit using a YM-30 membrane to a final volume of 1–2 ml. All purification steps were carried out at 4 °C. The purity of enzyme preparations was assessed by densitometry of silver-stained SDS-polyacrylamide gel, which indicated the presence of either a single protein species or a major band that constituted >90% of the total protein stained (data not shown).

**Enzyme Assays—**Trioleinase activity was measured using a triolein emulsion containing radiolabeled triolein. Triolein (7.5 mg), phosphatidylcholine (1 mg), and 50 μCi of [3H]triolaen were dried under nitrogen. A volume of 2.1 ml of 0.2 M Tris-HCl, pH 8.0, and 0.4% bovine serum albumin in 0.2 M Tris-HCl, pH 8.0, was added to the lipid mixture before it was sonicated on ice for 8 min at a 50% pulse. After sonication, 0.5 ml of 4% bovine serum albumin in 0.2 M Tris-HCl, pH 8.0, was added to the substrate mixture. A volume of 0.1 ml of the substrate was mixed with 0.1 ml of sample before being incubated at 37 °C for 1 h. The reaction was stopped by the addition of 3.25 ml of chloroform/methanol/heptane (1:25:1:1:1), and phases were separated by the addition of 1.05 ml of 0.1 M H2BO3 and 0.1 M K2CO3, pH 10.5. After 30 s, the mixture was centrifuged for 5 min at 1500 × g. A 1.0-ml aliquot of the upper phase was sampled for radioactive counting. ApoC-II-dependent lipase activity was determined by performing the assay in the presence of an apoC-II carboxyl-terminal fragment spanning residues 44–79 (apoC-II-(44–79)). This apoC-II fragment has been shown to have the same activating potential as intact apoC-II (15).

Phospholipase activity was measured as described previously (16) using dioleoylphosphatidylethanolamine containing 1-[3H]dioleoyl-sn-glycero-3-phosphothanolamine. Protein concentration was determined by the Bradford method (17).

**Electrophoresis and Immunoblotting—**Samples were mixed with 0.5 volume of buffer containing 2% SDS, 0.1 M Tris-HCl, pH 6.8, 5% glycerol, 10% β-mercaptoethanol, and 0.05% bromphenol blue. The mixture was placed in boiling water for 5 min prior to loading onto a 10% acrylamide gel. Gels were electroblotted onto nitrocellulose and subsequently blocked for 1 h in Tris-buffered saline containing 3% bovine serum albumin. After blocking, the membrane was incubated overnight at 4 °C with either a monoclonal antibody specific for human HL or a chicken polyclonal antibody raised against bovine LPL (a kind gift from O. Ben-Zeev). The monoclonal antibody was generated as described previously (18) from the fusion of P3Ag8.653.1 myeloma cells with the spleen of a BALB/c mouse immunized with purified human HL. Hybridomas secreting HL-specific antibodies were identified by solid-phase assays with HL-coated plates as well as by Western blotting. The antibody was purified from ascites fluid by chromatography on a Mono-Q anion-exchange column (Amersham Pharmacia Biotech). Immunoblotting with the monoclonal or polyclonal antibodies was detected with either anti-mouse IgG or anti-chicken IgG conjugated to biotin. After washing, the blot was incubated with streptavidin conjugated to horseradish peroxidase. The blot was developed with chemiluminescence reagents (Pierce) and exposed to x-ray film.

**Cross-linking ApoC-II-(44–79) to Lipase—**The photoreactive heterobifunctional cross-linker sulfo-succinimidyl 2-3′-dithiopropionate (SADP; Pierce) was radiolabeled and attached to apoC-II-(44–79). In a final volume of 0.1 ml of 20 mM sodium phosphate, pH 7.4, 20 nmol of SADP, 50 nmol of KI, and 100 μCi of Na125I were added to a precoated IODO-GEN® tube (Pierce). After a 15-min incubation in the dark, the samples were dialyzed against 20 mM sodium phosphate, pH 7.4, to remove unassociated SADP-apoC-II-(44–79). Samples were exposed to long-wave ultraviolet light for 3 min (dark controls were processed simultaneously) prior to analysis by SDS-PAGE.

**Heparin-Sepharose Chromatography—**Purified enzyme preparations were applied to a heparin-Sepharose column (1 × 5 cm) equilibrated in 0.35 M NaCl and sodium barbital, pH 7.2. After washing with equilibration buffer, each enzyme was eluted with a linear NaCl gradient (0.35–1.5 M) in 5 mM sodium barbital, pH 7.2. Fractions were assayed for lipase activity; mass was assayed by Western blotting; and the salt concentration was monitored by conductivity measurements.

**RESULTS**

To determine the immunoreactivity and to verify the identity of the chimeric lipases, purified recombinant enzymes were separated by SDS-PAGE, electrolabeled, and probed with either the anti-HL monoclonal antibody (A) or the anti-LPL polyclonal antibody (B). The migration positions of molecular mass standards (in kilodaltons) are indicated on the left. HL and the chimeric lipases were detected by the anti-HL monoclonal antibody, whereas HL-LPLC2 and LPL were recognized by the anti-LPL polyclonal antibody. The figure is representative of several experiments.

![Fig. 2. Western blot analysis of chimeric lipases.](image)

**C-terminal Subdomain Chimeric Lipases**

| HL-LPLC1 | HL-LPLC2 | LPL |
|----------|----------|-----|
| 64 kDa   | 66 kDa   | 56 kDa |

There is evidence to indicate that the interaction of LPL with apoC-II involves a specific protein-protein interaction (19). To determine if such an interaction existed between apoC-II-(44–79) and HL-LPLC2, the photoreactive cross-linker SADP was radiolabeled with Na125I and attached to apoC-II-(44–79) be-
C-terminal Subdomain Chimeric Lipases

Purified recombinant lipases were incubated with a triolein emulsion under either low or high salt conditions and in the presence or absence of apoC-II-(44–79) as indicated. Enzyme activity was measured as described under “Experimental Procedures” and is expressed as the specific activity of each enzyme in the presence of 0.15 M NaCl, which was assigned a value of 1.0. The data are presented as the mean ± S.D. of three independent measurements. The 100% values for HL, HL-LPLC1, HL-LPLC2, and LPL were 72 ± 6, 69 ± 1, 69 ± 7, and 69 ± 0.1, respectively.

| Lipase          | 0.15 M NaCl | 1 M NaCl | ApoC-II | ApoC-II + 1 M NaCl |
|-----------------|-------------|----------|---------|-------------------|
| HL              | 1.0         | 1.0 ± 0.2| 0.9 ± 0.2| 1.0 ± 0.1         |
| HL-LPLC1        | 1.0         | 0.8 ± 0.2| 1.0 ± 0.1| 0.9 ± 0.3         |
| HL-LPLC2        | 1.0         | 0.9 ± 0.1| 2.9 ± 0.7| 2.3 ± 0.4         |
| LPL             | 1.0         | 0.3 ± 0.1| 6.9 ± 0.7| 4.0 ± 1.5         |

FIG. 3. Cross-linking apoC-II-(44–79) with LPL and HL-LPLC2.

The photoreactive cross-linker SASD was radiolabeled with Na125I and covalently bound to apoC-II-(44–79) before it was subjected to photolysis in the presence of equimolar amounts of purified lipase as described under “Experimental Procedures.” Samples were subjected to SDS-PAGE, and the gel was digitized by a PhosphorImager. The migration positions of proteins with known molecular masses (in kilodaltons) are indicated.

TABLE II

Phospholipase activity of chimeric lipases

Purified recombinant lipases were incubated with a phospholipid emulsion under low salt conditions and in the presence or absence of apoC-II as indicated. Specific enzyme activity was measured as described under “Experimental Procedures” and is expressed as nmol/min/μg. The data are presented as the mean ± S.D. of three independent measurements.

| Lipase          | 0.15 M NaCl | 0.15 M NaCl + apoC-II |
|-----------------|-------------|----------------------|
| HL              | 44 ± 7      | 21 ± 6               |
| HL-LPLC1        | 15 ± 1      | 14 ± 1               |
| HL-LPLC2        | 37 ± 4      | 39 ± 5               |
| LPL             | 4 ± 1       | 3 ± 1                |

DISCUSSION

In the absence of crystal structure information, molecular biology techniques have been applied to investigate the structure-function relationships of HL and LPL. The construction of chimeric lipases differs from other approaches because the objective is to create structurally altered, but active, lipase molecules. The preservation of catalytic activity following the engineered change ensures that the tertiary structure of the molecule was not significantly altered. Moreover, the engineered change can be evaluated within the context of a functional lipase, so the subsequent conclusions are substantially strengthened.

The information gained from several structural and functional studies of both lipases has led to the proposal of a model for an active dimeric enzyme (6, 24). This model predicts that two monomer subunits are arranged in a head-to-tail arrangement such that the carboxyl-terminal domain of one subunit is juxtaposed in close proximity to the amino-terminal domain of the opposing subunit. We have recently provided evidence to support a head-to-tail subunit arrangement for LPL through the expression of a catalytically active tandem repeat of two LPL monomers (25). We hypothesize that the initial interaction of the enzyme with lipid substrates is facilitated by contact with the carboxyl-terminal domain, which enables the catalytic reaction to take place at the active site found within the neighboring amino-terminal domain originating from the other sub-
unit. This is supported by several studies, including the inhibition of lipolytic, but not esterolytic, activity of chimeric lipases by carboxyl terminus-specific antibodies (6, 7); the impaired lipid binding of a proteolytically truncated LPL molecule (8); and the retention of 70% of the esterolytic activity, but only 6% of the lipolytic activity of an LPL molecule in which tryptophans at positions 393 and 394 were replaced with alanines (9). Also, several reports have identified the carboxyl-terminal domain as containing regions that contribute to the heparin affinity of these lipases (6, 7, 10, 13, 28, 29).

To further localize the carboxyl-terminal domain functions of HL and LPL, we constructed and analyzed new chimeric lipases in which portions of the carboxyl-terminal domain were exchanged between the lipases (Fig. 1). The HL-LPLC1 chimera consisted of the first 344 residues of human HL joined to residues 331–388 of human LPL, which then terminated with residues 415–476 of human HL. The second chimera, HL-LPLC2, included the first 414 residues of human HL and terminated with the last 60 amino acids of human LPL. Both chimeras were detected by a monoclonal antibody specific for human HL, indicating that the recognized epitope was contained within the first 344 residues of human HL. However, only HL-LPLC2 could be recognized with the anti-LPL polyclonal antibody, confirming its chimeric nature (Fig. 2). The inability to detect HL-LPLC1 may be a consequence of the specificities of the antibodies in the polyclonal serum or the inaccessibility of the first portion of the carboxyl-terminal domain to antibody.

Both chimeric lipases were lipolytically active, able to catalyze triolein emulsions to a similar extent as compared with HL and LPL, and thus were likely to be active dimeric proteins. Despite differences in the composition of the carboxyl-terminal domain, the lipid affinity of these chimeric lipases did not appear to be significantly affected. As mentioned previously, the tryptophan cluster composed of residues 390, 393, and 394 of LPL is thought to contribute to the lipid binding properties of this lipase (9). These residues were all present in the HL-LPLC2 chimera, which may account for its ability to catalyze lipid substrates. However, only one of these tryptophan residues was conserved in the analogous HL sequence (residue 416). There were two additional tryptophan residues at positions 403 and 407 of the native HL sequence that are not found in LPL, but these residues were not present in the HL-LPLC1 chimera. Consequently, the region or residues responsible for the lipid affinity of HL-LPLC1, and thus of HL, remain to be better defined. Additional studies comparing the relative lipid affinity of these chimeric enzymes may help to further localize this function.

The ability of HL-LPLC2 to be activated by apoC-II-(44–79), a carboxyl-terminal fragment of apoC-II that has been shown to have the same activating potential as the intact apolipoprotein (15), demonstrated for the first time that a region within the LPL carboxyl-terminal domain is important in cofactor activation of LPL. In support of this proposition, apoC-II-(44–79) was shown by cross-linking studies (Fig. 3) to have a specific protein-protein interaction with LPL and HL-LPLC2, but not with HL or HL-LPLC1, consistent with the effect of the cofactor fragment on lipolytic activity (Table I). However, no apo-C-II activating properties were observed when a phospholipid emulsion was used as substrate. In fact, the relative specific activity of the chimeric enzymes was not appreciably changed in comparison with HL. Earlier reports (23, 26) have indicated that regions within the amino-terminal domain, specifically a surface loop or lid domain, determine to a large extent the relative phospholipase activity of each lipase.

The finding that the 60 carboxyl-terminal amino acids of LPL participate in apoC-II activation is in apparent variance with previous studies that have suggested that a region in the
amino-terminal domain of the molecule was responsible for cofactor activation (7, 10). However, these findings may not be contradictory. Instead, the combined findings support the notion that apoC-II interacts simultaneously with regions located within the amino- and carboxyl-terminal domains of opposing subunits that make up an LPL dimer. One consequence of the head-to-tail subunit models for HL and LPL (10, 24, 27) is that the amino- and carboxyl-terminal domains of opposing subunits are in close proximity, allowing interdomain and inter-subunit interactions during the catalysis of lipid substrates. The observation that the magnitude of activation (3-fold) for HL-LPLC2 by apoC-II-(44–79) was lower than for native LPL (7-fold) suggested that additional regions were responsible for apoC-II activation as well. In addition, it is noteworthy that apoC-II was able to achieve only 25% of the maximal activity for a proteolytically truncated bovine LPL (terminating at residue 390) compared with intact LPL (8). Also, an LPL chimera made up of residues 1–335 of human LPL and residues 330–476 of human HL was stimulated only 3-fold by apoC-II compared with 10-fold for native LPL (7), suggesting that regions within the N-terminal domain alone were not sufficient to achieve optimal activation. Taken together, these studies are consistent with the hypothesis that apoC-II interacts simultaneously with regions in both the amino- and carboxyl-terminal domains of HL and LPL, i.e. LPL has a higher affinity (1.3 versus 0.75 μM) than HL. The heparin affinity of the chimeras was determined by the parental lipase that contributed the last 60 amino acids to the molecule. These data strongly supported the view that regions within the carboxyl-terminal domains of HL and LPL were solely responsible for their relative heparin affinities, although this did not exclude the possibility of the presence of an additional heparin-binding region within the amino-terminal domain. However, if an amino-terminal heparin-binding site exists, it plays a minor role in overall binding and cannot explain the binding difference between the two lipases. Analysis of the heparin affinity of these chimeric lipases and identification of the charged residues indicated that the final 60 carboxy-terminal amino acids of HL and LPL were critical in determining lipase heparin affinity, confirming earlier predictions based on amino acid sequence alignment of LPL, HL, and pancreatic lipase (10). These results are also in agreement with a recent report identifying residues 404–430 of LPL as a heparin-binding site (13, 28, 29). Consistent with a putative heparin-binding domain, the final 60 carboxy-terminal residues contain 13 lysine or arginine residues for each lipase. These findings considered collectively indicate that the major determinant of lipase heparin binding is in the last 60 amino acids of the molecule.

In summary, we have constructed and analyzed lipolytically active carboxyl-terminal subdomain chimeras of HL and LPL. The chimeras were composed primarily of human HL in which different portions of the carboxyl-terminal domain were replaced with the corresponding sequence from human LPL. The chimera containing the last 60 amino acids of LPL (HL-LPLC2) was responsive to apoC-II activation and could be specifically cross-linked to this apolipoprotein, indicating that this region of LPL was involved in apoC-II stimulation. In addition, the relative heparin affinity of each chimera strongly suggested that a carboxyl-terminal heparin-binding domain likely exists within the terminal 60 residues of each lipase. Investigation of the structure-function relationships of lipases through the construction and analysis of chimeras continues to provide valuable information that will enable a better description of the specific mechanisms of action of these key enzymes in lipid metabolism.

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