Functional characterization of a chimeric lipase genetically engineered from human lipoprotein lipase and human hepatic lipase

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Abstract Lipoprotein lipase (LPL) and hepatic lipase (HL) mediate the hydrolysis of triglycerides and phospholipids present in circulating lipoprotein particles and are essential for normal lipid metabolism. Both enzymes have a similar primary amino acid structure and share requirements for intact catalytic, lipid binding, and heparin binding domains. However, LPL and HL exhibit different substrate specificities and cofactor requirements. In order to characterize the functional domains necessary for LPL activity, a chimeric lipase consisting of the amino-terminal 314 amino acids of human LPL and the carboxyl-terminal 146 amino acids of human HL was synthesized by joining the cDNA of both lipases at the 5'-end of exon 7. Northern blot hybridization and Western blot analyses revealed the size of the chimera mRNA and protein to be approximately 1.5 kb and 55 kDa, respectively. The chimeric enzyme hydrolyzed both long chain and short chain fatty acid triacylglycerols and had catalytic properties that were similar to lipoprotein lipase. Thus, apolipoprotein (apo)C-II was required for maximal lipase activity, and high salt concentration abolished the ability of the chimera to hydrolyze triolein even in the presence of apoC-II. A monospecific anti-HL polyclonal antibody interacting with the C-terminal HL-derived domain of the chimeric enzyme abolished the enzyme's ability to hydrolyze triglyceride emulsion but not tributyrin substrates. Analysis of the heparin binding properties of the chimeric enzyme using heparin-Sepharose affinity chromatography revealed an elution pattern which was intermediate between that of lipoprotein and hepatic lipase. In summary, we have characterized the functional properties of an LPL-HL chimeric enzyme. Our studies indicate that the LPL-derived NH_2-terminal domain is the site of interaction between apoC-II and LPL and determines the catalytic properties of the chimera, whereas the HL-derived C-terminal domain plays a major role in mediating the lipase interaction with long chain triacylglycerol substrates. In addition, both the LPL-derived NH_2-terminal domain and the HL-derived C-terminal domain contribute to the heparin binding properties of the LPL-chimera.—**Dichek, H. L., C. Parrott, R. Ronan, J. D. Brunzell, H. B. Brewer, Jr., and S. Santamarina-Fojo.** Functional characterization of a chimeric lipase genetically engineered from human lipoprotein lipase and human hepatic lipase. *J. Lipid Res.* 1993. **34:** 1393–1401.

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Lipoprotein lipase (LPL) and hepatic lipase (HL) catalyze the hydrolysis of triglycerides present in plasma lipoproteins to free fatty acids (FFA) and mono- and diacylglycerols and are, thus, essential for normal lipid metabolism (1, 2). As a member of the lipase family, LPL shares a high degree of primary amino acid sequence homology with HL and pancreatic lipase (PL) (3–5). Recently, the three-dimensional structure of human (h) PL has been established by X-ray crystallography (6). Based on these studies hPL can be partitioned into two major structural domains: a larger NH_2-terminal domain consisting of amino acids 1–335 joined by a small spanning region to the 111 amino acid C-terminal domain. The NH_2-terminal domain contains the catalytic site required for hydrolysis of triglycerides. Because of the high degree of primary amino acid sequence homology between hPL, hLPL, and hHL as well as the conservation of disulfide bonds, it is hypothesized that the three-dimensional structures of hLPL and hHL are similar to that of hPL. However, despite their structural similarities, there are some basic functional differences among the three lipases. Thus, LPL exerts its hydrolytic action on triglycerides associated with very low density lipoproteins (VLDL) and chylomicron particles, whereas HL hydrolyzes triglycerides contained in intermediate density lipoproteins (IDL) and chylomicron remnant particles (2, 7, 8). In addition, HL acts as a phospholipase facilitating the conversion of HDL_2 to HDL_3 (9). Both LPL and HL are anchored by glycosaminoglycans to the capillary endothelium and are released into the circulation by intravenous administration of heparin (1, 2). LPL requires dimerization (10) and

Abbreviations: LPL, lipoprotein lipase; HL, hepatic lipase; PL, pancreatic lipase; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; apoC-II, apolipoprotein C-II; FFA, free fatty acids; nmol, nanomoles.

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a cofactor, apoC-II for activation, whereas HL is active in the absence of a cofactor (2, 11, 12).

LPL consists of at least five functional domains that are necessary for the enzyme to fulfill its metabolic function. In hLPL, these include a hydrolytic domain containing the catalytic triad residues Ser^{132}-Asp^{156}-His^{241} which have been recently identified as the active site residues by site-directed mutagenesis (13, 14), as well as an interfacial lipid recognition site initially proposed to consist of a hydrophobic segment spanning amino acids 126 to 135 (5). Since the corresponding residues (amino acids 146 to 155) in hPL are buried deep within the catalytic pocket and are, thus, inaccessible to the substrate, amino acids 126 to 135 of hLPL are unlikely to be involved in the process of interfacial activation (6). Other recent studies (15–17) indicate that the C-terminal domain of hLPL may play a significant role in lipid substrate interaction. The location of a third important functional domain of hLPL, the heparin binding site, which may mediate its interaction with the glycosaminoglycan structures in the endothelial wall, is yet to be definitively defined. Analysis of the primary amino acid sequence of hLPL reveals three regions rich in basic residues including: 260 to 267 (unique to LPL), 292 to 306, and 441 to 446 which could potentially be involved in heparin binding. Based on previous studies involving proteolytic digestion of LPL with trypsin and chymotrypsin, the basic residues located within the C-terminal region of hLPL bordered by residues 229 and 388 may be the most important in heparin binding (18, 19). To date, very little is known about two other important functional domains of hLPL: the site of interaction with its apoC-II cofactor and the site of dimer formation.

HL, like LPL, has been proposed to contain a catalytic domain which consists of the active residues Ser^{145}-Asp^{171}-His^{256}. Site-directed mutagenesis of Ser^{147} of rat HL (20) has confirmed the importance of this residue for enzyme activity. Two potential interfacial lipid recognition sites, 138 to 147 and 259 to 268, as well as a heparin binding site at residues 467 to 476 have also been postulated (4, 21). Human HL, however, contains other areas rich in basic amino acid residues including 312 to 321 and 428 to 433, which could also participate in heparin binding.

In order to further characterize these important functional domains of the two human heparin-releasable lipases, we constructed a human chimeric lipase (LPL-chimera), where the structural NH_2-terminal domain is derived from the amino-terminal 314 amino acids of hLPL and the C-terminal domain consists of the last 146 amino acids of hHL. Our studies localize the site of apoC-II/LPL interaction to the NH_2-terminal domain of LPL and indicate an important role of the C-terminal domain of LPL, HL, and LPL-chimera in lipid–substrate interaction.

EXPERIMENTAL PROCEDURES

Cloning of human hepatic lipase and human lipoprotein lipase cDNA

A probe generated by PCR amplification of exon 2 of HL (22) was used to screen a lambda gt11 human liver cDNA library (kind gift from Philip Leder, Harvard University). Human hepatic lipase cDNA was amplified using the polymerase chain reaction and primers incorporating restriction sites for Xba I and Hpa I, (5'-CTT CAG AAA TTA TCT AGA AAG CCT GGA CCC-3' and 5'-ATT CAT TTA TTC GTT AAC CTG GGT CTT CAT-3'). The amplified sequence, including 28 nucleotides upstream of the translational start site of hHL and 19 nucleotides downstream of the stop codon (4, 22), was digested with Xba I and Hpa I and cloned into the pUC18-based pCMV expression vector (23). The hHL nucleotide sequence was confirmed by DNA sequencing using the dideoxynucleotide chain termination method (24). Cloning of hLPL has been previously described (23).

Construction of the human LPL-chimera

The chimeric enzyme was generated by the splicing overlap extension method (25) using the splicing primers: (HL 13 5'-CCC TAC AAA GTC TTC-CAT TAC CAG TTA AAG ATC CAG-3' and LPL 14 5'-CTT TAA CTG GTA ATG-GAA GAC TTT GTA GGG CAT CTG-3') with primer sequences to the 5' and 3' ends of the vector, respectively. The amplified fragment containing the DNA sequence coding for the 314 N-terminal amino acids of hLPL and C-terminal 146 residues of hHL was then subcloned into the pUC18-based expression vector, pCMV, and used in transient transfection studies as described (23, 26). The constructs pCMV-LPL, pCMV-HL, and pCMV-LPL-chimera were characterized by DNA sequencing prior to transfection into human embryonal kidney 293 cells (ATCC, Rockville, MD) (27).

Expression studies

DNA transfection was performed using the calcium-phosphate co-precipitation method (26) modified by supplementing the media with 2 units/ml of heparin (Lyphomed, Inc., Rosemont, IL) to stabilize the LPL, as described (28). Media was harvested 42 h after transfection and stored at −70°C after addition of glycerol to a final concentration of 30%. Intracellular protein was isolated as described (29). Media and intracellular lipase activities were quantitated in triplicate (30).

Northern blot hybridization analysis of RNA

Northern blot hybridization analysis was performed on 20 μg of total RNA isolated from two 100-mm cell culture dishes of 293 cells transfected with pCMV-LPL, pCMV-HL, or pCMV-LPL-chimera. The RNA was separated

by 1.1% agarose gel electrophoresis at 25 V for 16 h in the presence of 6% formaldehyde followed by transfer to a Nytran membrane (Schleicher & Schuell) (31). Hybridization was performed by using either a full-length hLPL cDNA probe or a full-length hHL cDNA probe as described previously (23, 32). The gels were stained with ethidium bromide to confirm that equivalent amounts of RNA were electrophoresed in each lane.

Western blot analysis of media lipase

Western blot analysis was performed on serum-free conditioned media from cells transfected with pCMV-control plasmid, pCMV-LPL, pCMV-HL, and pCMV-LPL-chimera after 42–48 h incubation (33). Media were dialyzed against four changes of 4 l of 0.01 M ammonium bicarbonate (pH 8.2) for 8 h at 4°C and 1-ml aliquots were dried in a Speedvac. Two 1-ml lyophilized media aliquots were combined in 30 μl sample buffer (1.5 M Tris, 4% $NaDodSO_4$, 8% sucrose, 2% Bromophenol blue) heated at 95°C for 3 min, prior to loading on a 12% SDS-PAGE gel. Electrophoresis was conducted at 200 V for 1.5 h followed by transfer to an Immobilon-P membrane (Millipore, Bedford, MA) at 600 mA for 3 h. Blocking was performed with 3% BSA in PBS for 2 h followed by overnight incubation at room temperature with either a goat anti-hHL antibody (1:200 dilution) (8), or a chicken anti-bLPL antibody (1:200 dilution) (34), both kindly provided by Dr. Ira Goldberg. Immunodetection of lipase was performed as described in the Vectastain ABC kit (Vector Laboratories, Inc., Burlingame, CA).

Quantitation of lipase activity

Total lipolytic activity in both cells and media was determined in triplicate as previously described using radiolabeled glycerol [1-^{14}C]trioleate (30). Lipase studies were performed in the presence or absence of apoC-II and 1 M NaCl final concentration. Esterase activity in the media was quantified in triplicate with radiolabeled tributyrin (35). Selective inhibition of LPL and HL was determined after incubating samples with LPL or HL specific antibodies, respectively, for 4 h on ice prior to lipase activity quantitation. Antibodies used included the caprylic acid- and ammonium sulfate-precipitated IgG fraction of the monoclonal anti-bovine LPL antibody 5D2, directed to residue 400 in the C-terminal region of the enzyme (36, 37), a goat anti-human HL (kindly provided by Dr. Ira Goldberg) (8) as well as a rabbit polyclonal antibody raised against the NH_2-terminal 29 residues of human LPL. The anti-HL antibody and the antibody against the NH_2-terminal end of hLPL where further purified using Immunopure (A/G) IgG Purification Kit (Pierce, Rockford, Il.).

Heparin-Sepharose affinity chromatography

Affinity chromatography of conditioned media from cells transfected with hLPL, hHL, and LPL-chimera was performed as described by Emi et al. (38) with some modifications. A heparin-Sepharose CL-6B (Pharmacia-LKB Biotechnology, Uppsala, Sweden) column, 0.7 cm × 2.5 cm, was equilibrated at 5°C with 0.4 M NaCl in 0.01 M sodium phosphate (pH 7.4), 0.1% CHAPS, and 30% glycerol. The transfected lipase was applied to the column at 6 ml/h. The unabsorbed protein was eluted with 10 ml of the equilibration buffer. The lipase was eluted from the column with a 20 ml linear gradient (10 ml of equilibration buffer and 10 ml of 2 N NaCl in 0.01 M Na sodium phosphate, pH 7.4, 0.1% CHAPS, and 30% glycerol). The sodium chloride concentration in the column fractions was monitored with a Radiometer conductivity meter at 5°–7°C. Only alternating fractions were monitored in order to reduce cross contamination. The conductivities were then related to a standard curve of NaCl concentrations. One-ml fractions were collected in glass tubes containing 2 units of heparin and the fractions were stored at −70°C.

RESULTS

A schematic of the LPL-chimera generated by joining the nucleotide sequence coding for the N-terminal 314 amino acids of hLPL to that coding for the C-terminal 146 amino acids of hHL is illustrated in **Fig. 1.** The junction was made to coincide with the short spanning region between the two structural domains of the lipases (1, 2, 6). The catalytic triad residues of the LPL-chimera Ser^{132}-Asp^{156}-

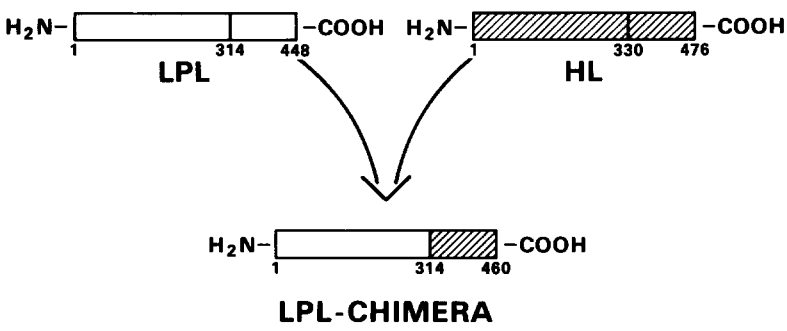

Fig. 1. Schematic representation of the LPL-chimera. Nucleotide sequences coding for the 314 amino-terminal residues of hLPL and the 146 carboxyl-terminal residues of hHL were linked using the overlap extension method. The resulting chimera consists of 460 amino acid residues.

His[241] and one potential N-glycosylation site, Asn[43], were derived from hLPL, whereas basic regions potentially involved in heparin binding were derived from both hLPL (residues 260–267 and 292–306) and hHL (residues 467–476) sequences. Two additional potential glycosylation sites Asn[339] and Asn[374] were derived from hHL. Thus, compared to hLPL and hHL, which contain two and four N-glycosylation sites, respectively, the LPL-chimera has a total of three potential sites for N-glycosylation.

Northern blot hybridization analysis of total cellular RNA isolated from human embryonal kidney 293 cells transfected with pCMV-LPL, pCMV-HL, and pCMV-LPL-chimera is shown in **Fig. 2.** The LPL-chimera mRNA was of a size similar to that of LPL and HL (~1.5 kb) and hybridized with both the hLPL and the hHL cDNA probes.

Fig. 3 contains a Western blot analysis of media from cells transfected with plasmids containing control (the pCMV vector), LPL, HL, and LPL-chimera cDNA. The media in lanes 1–4 were incubated with a chicken anti-LPL antibody whereas those in lanes 5 and 6 were incubated with a goat anti-hHL antibody. The LPL-chimera (lanes 4 and 6), visualized with both the anti-LPL antibody and the anti-HL antibody, is of a slightly smaller apparent molecular weight than either of the two parent lipases, possibly resulting from different postranslational processing of the LPL-chimera. Densitometric analysis of this and similar Western blots revealed that the abundance of the LPL-chimera in the conditioned media was approximately 25% of that of hLPL (data not shown).

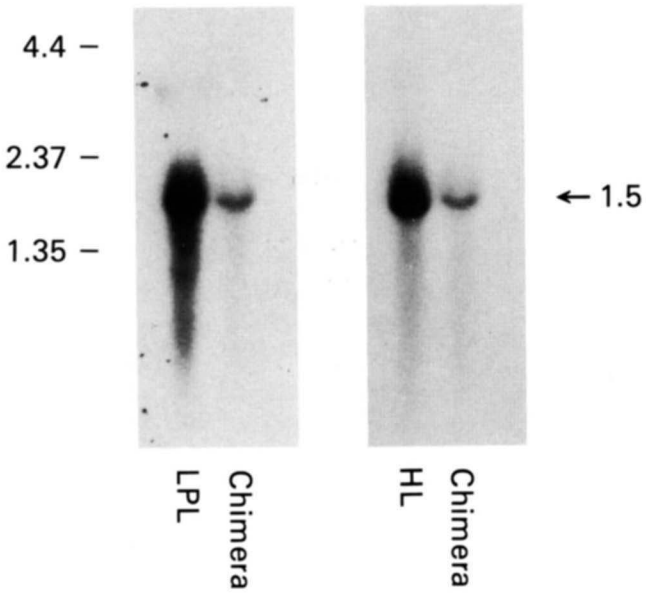

Fig. 2. Northern blot hybridization analysis of 20 μg total RNA isolated from human embryonal kidney 293 cells transfected with constructs containing normal human LPL, human HL, or LPL-chimera. The RNA was separated by 1.1% agarose gel electrophoresis in the presence of 6% formaldehyde. Molecular weight markers are indicated on the left. The molecular weight of the mRNA is indicated by the arrow on the right.

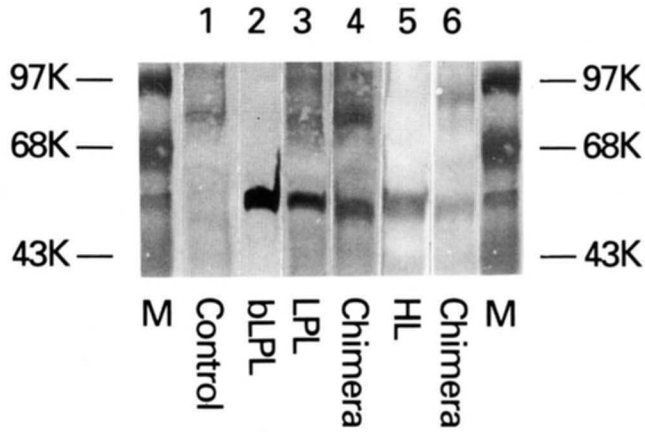

Fig. 3. Western blot analysis of media from human embryonal kidney 293 cells transfected with control (pCMV-) (lane 1), hLPL (lane 3), hHL (lane 5), or LPL-chimera (lanes 4 and 6) plasmids. Two ml of transfection media was loaded in each of lanes 1, 3, 4, 5, and 6. One μg of bovine LPL standard was loaded in lane 2. The antibodies used were chicken anti-bovine LPL in lanes 1–4 and goat anti-human HL in lanes 5 and 6. Molecular weight markers are shown on right and left.

In order to characterize the hydrolytic properties of the LPL-chimera, media from transfected cells were assayed for lipase activity using the emulsified substrate, triolein (**Table 1**). Lipase assays were performed in the presence and absence of apoC-II, as well as of a high salt (1 M NaCl) concentration. Like human LPL, the LPL-chimera required apoC-II for optimal activity and was inhibited by a high salt concentration. As with native plasma hHL, expressed hHL did not require a cofactor for activation and was not salt-sensitive. Lipase activity was also determined using the short chain fatty acid, monodisperse triglyceride, tributyrin (Tables 2, 3, and 4). Unlike the emulsified substrate, triolein, tributyrin hydrolysis is independent of lipid interaction and reflects the esterase properties of the lipase.

As expected, a monoclonal LPL-antibody directed to the C-terminal region of the enzyme had no effect on HL or LPL-chimera activities (illustrated in **Table 2**). This

TABLE 1. Lipase activity in the media of transfected cells[a]

Plasmid	Without ApoC-II	With ApoC-II	1 M NaCl + ApoC-II
LPL	6.8 ± 0.8	70.0 ± 6.9	1.9 ± 0
HL	18.7 ± 1.4	16.5 ± 0.8	14.0 ± 1.5
LPL-chimera	9.3 ± 1.4	31.1 ± 3.9	2.8 ± 0.6

[a]Activity expressed in nmol FFA/ml per min.

TABLE 2. Lipase activity in the media of transfected cells[a]: effect of LPL-antibody[b]

Plasmid	Triolein[c]	Triolein[c] + anti-LPL	Tributyrin	Tributyrin + anti-LPL
LPL	95.0 ± 2.9	7.1 ± 0.4	2.3 ± 0.2	1.6 ± 0.3
HL	22.1 ± 2.5	20.6 ± 1.6	11.3 ± 0.2	12.1 ± 1.0
LPL-chimera	33.3 ± 3.6	27.6 ± 3.5	1.2 ± 0	1.3 ± 0.1

[a]Activity expressed in nmol FFA/ml per min.
[b]Monoclonal antibody directed against the C-terminal end of LPL.
[c]Triolein assay performed in the presence of apoC-II.

antibody abolished the ability of LPL to hydrolyze emulsified triolein but not the monodisperse tributyrin substrate.

In contrast, incubation with a polyclonal antibody specific for HL abolished the ability of both HL and LPL-chimera (containing the C-terminus of HL) to hydrolyze triolein. Relative to the inhibition observed with both HL and LPL-chimera using the emulsified triolein substrate, minimal effects were observed using the water-soluble substrate, tributyrin (**Table 3**). As expected, this HL-specific antibody did not inhibit LPL. These two studies indicate that antibodies interacting with the C-terminal domain of any of the three lipases interfered with the ability of these enzymes to hydrolyze emulsified substrates without having a significant effect on the hydrolysis of monodisperse, water-soluble substrates. The specificity of this inhibition was demonstrated by performing similar studies with a polyclonal rabbit antibody directed to residues 1–29 at the NH_2-terminal end of human LPL with a titer similar to that of the monoclonal anti-bLPL antibody. Incubation of this antibody with LPL, HL, or LPL-chimera had no effect on their ability to hydrolyze either substrate (**Table 4**).

Interestingly, when the ratio of triolein to tributyrin hydrolysis in all three experiments (Tables 2, 3, and 4) are calculated for LPL, HL, and LPL-chimera (37.7 ± 11.8, 3.1 ± 1.0, and 24.6 ± 3.9, respectively) the substrate hydrolysis for the LPL-chimera parallels that of LPL and not that of HL.

A comparison of the heparin binding properties of hLPL, hHL, and the LPL-chimera in conditioned media from transfected human embryonal kidney 293 cells was performed using heparin-Sepharose affinity chromatography. Protein was eluted with a linear gradient from 0.4 M NaCl to 2.0 M NaCl and the column fractions were assayed for lipase activity (**Fig. 4**). As expected, LPL activity peaked at 1.3 M NaCl, whereas HL peaked at 0.75 M NaCl. The chimera lipase activity peaked at 0.9 M NaCl, a salt concentration intermediate between that of HL and LPL. Similar findings were obtained when the elution of the LPL-chimera in a heparin-Sepharose column was analyzed by block elution (data not shown).

DISCUSSION

We have expressed a functional human chimeric enzyme, designated LPL-chimera, consisting of the 314 amino-terminal amino acids of hLPL and the 146 carboxyl-terminal amino acids of hHL. The LPL-chimera contains the catalytic triad Ser^{132}-Asp^{156}-His^{241} (13) and two potential heparin binding sites rich in basic residues (292–306) (19) and (260–267) of hLPL. Additionally the LPL-chimera contains the asparagine 43 N-glycosylation site, previously reported to be essential for LPL secretion and activation (39). From human HL, the LPL-chimera retains two potential N-glycosylation sites (Asn^{339} and Asn^{374}) (4) and a stretch of basic amino acid residues potentially involved in heparin binding KSKTSKRKIR (467–476) (21). Thus, the LPL-chimera contains potentially important structural domains derived from both LPL and HL.

TABLE 3. Lipase activity in the media of transfected cells[a]: effect of HL-antibody[b]

Plasmid	Triolein[c]	Triolein[c] + anti-HL	Tributyrin	Tributyrin + anti-HL
LPL	71.4 ± 16.9	76.8 ± 7.9	2.9 ± 0.1	2.8 ± 0.4
HL	14.1 ± 0.7	0.3 ± 0	3.4 ± 0.3	2.5 ± 0.3
LPL-chimera	22.3 ± 3.6	2.4 ± 0.4	1.1 ± 0.1	1.2 ± 0.1

[a]Activity expressed in nmol FFA/ml per min.
[b]Polyclonal anti-human HL antibody.
[c]Triolein assay performed in the presence of apoC-II.

TABLE 4. Lipase activity in the media of transfected cells[a]: effect of N-terminal LPL-antibody[b]

Plasmid	Triolein[c]	Triolein[c] + anti-LPL[b]	Tributyrin	Tributyrin + anti-LPL[b]
LPL	176 ± 48.7	218 ± 33.7	3.7 ± 03	4.3 ± 0.6
HL	47.0 ± 11.3	28.9 ± 3.8	14.2 ± 0.2	14.4 ± 0.6
LPL-chimera	41.0 ± 12.8	31.5 ± 1.1	1.6 ± 0.2	1.2 ± 0.2

[a]Activity expressed in nmol FFA/ml per min apoC-II.
[b]Polyclonal antibody directed against residues 1–29 of human LPL.
[c]Triolein assay performed in the presence of apoC-II.

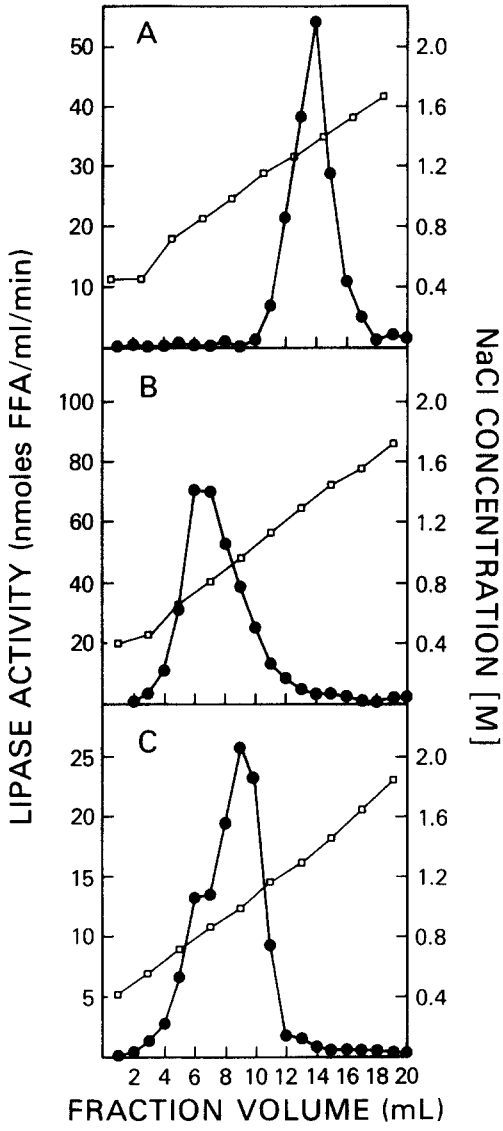

Fig. 4. Heparin-Sepharose elution profiles of media from human embryonal kidney 293 cells transfected with constructs containing normal human LPL (panel A), human HL (panel B), or LPL-chimera (panel C). The elution with phosphate buffer containing increasing concentrations of NaCl is illustrated. Lipase activity using triolein as substrate is indicated in nmol FFA/min per ml on the left. The sodium chloride concentration in the fractions was monitored with conductivity measurements and is indicated on the right as mol/l.

Functional characterization of the LPL-chimera indicates that it has catalytic properties similar to LPL. Like LPL it is salt-sensitive and requires apoC-II for activation. Because the salt sensitivity and apoC-II binding requirements are conferred by the amino-terminal LPL-derived portion of the chimera, this localizes the site of LPL-apoC-II interaction to the first 314 amino acids of hLPL. These 314 residues include the Lys^{147}-Lys^{148} sequence proposed by Yang et al. (40) to be involved in the interaction between LPL and apoC-II by forming salt bridges with the glutamic acid residues 78 and 79 of apoC-II. Interestingly, our data indicate that activation of LPL by apoC-II was several-fold greater than the apoC-II-mediated increase in LPL-chimera activity, an observation which may reflect alterations in the overall tertiary or quartenary structure of the chimera induced by the presence of the C-terminal domain of HL. Our findings of apoC-II activation of the LPL-chimera are consistent with the studies of Davis et al. (16) in which a similar chimeric lipase, consisting of residues 1–312 of human LPL and residues 330–472 of rat HL, was also reported to be salt-sensitive and dependent on apoC-II for activation whereas the converse chimera, consisting of the NH_2-terminal residues 1–329 of rat HL and the C-terminal residues 313–448 of human LPL, had a lipase activity that was both independent of apoC-II and salt-resistant (15). Thus, despite the species difference and the different construction of the chimeras, conclusions regarding the localization of this important functional domain of LPL were similar for both studies.

Further characterization of the LPL-chimera was performed by inhibition studies using antibodies specific for HL. Incubation with an anti-HL antibody demonstrated that interaction with the HL-derived C-terminal domain of the chimeric lipase abolished the ability of the enzyme to hydrolyze the emulsified substrate, triolein, but had no effect on tributyrin hydrolysis. Tributyrin, a short chain fatty acid triglyceride, can be used to determine esterase activity of the lipases independent of lipid interaction, thereby reflecting the catalytic function of the enzyme (35). In our study, antibody bound to the C-terminal domain of the LPL-chimera abolished hydrolysis of emulsified substrates but did not prevent access of the

short chain fatty acid triglycerides to the catalytic pocket located in the NH_2-terminal portion of the chimeric lipase or disrupt the esterase function of the lipase.

A possible explanation for these results is that the C-terminal domain of both HL and LPL is involved in the initial interaction of the enzyme with its lipid substrates, inducing a conformational change that opens up a 22-residue "lid" bordered by two disulfide bridges which covers the hydrolytic pocket. The proposed conformational change would expose a large hydrophobic area that allows access of the substrate to the catalytic site. This change in conformation may be prevented by antibodies bound to the C-terminal region of the enzyme. Monodisperse substrates, like tributyrin, however, may be able to access the catalytic site directly without movement of the LPL or HL "lid." A similar mechanism has been demonstrated for an unrelated, fungal lipase (41, 42), where a conformational change of the "lid" exposes the catalytic pocket of the enzyme. Unlike LPL and HL, however, the fungal lipase lacks a C-terminal domain that in hPL consists primarily of a β-pleated sheet structure (6, 43). Recently, Timmins, Poliks, and Banaszak (44) reported evidence for the involvement of β-pleated-sheet structure in the hydrophobic interaction with lipid in the lipovitellin-phospholipid complex. Thus, our studies are consistent with an important role of the C-terminal domain in lipid interaction. Analysis of their HL-LPL-chimeras have led Wong et al. (15) and Davis et al. (16) to a similar conclusion.

Another potential explanation for the observed antibody inhibition of the lipase activity of the LPL-chimera involves antibody disruption of the interaction between LPL and apoC-II. Since our studies localize the site of interaction between LPL and apoC-II to the NH_2-terminal domain of the lipase, it is highly unlikely that the C-terminal specific monoclonal anti-bLPL antibody (Table 2) mediates inhibition of triolein hydrolysis by disruption of apoC-II binding to LPL. Antibody-induced dissociation of the lipase dimer, (15), or alteration of the three-dimensional conformation of the enzyme induced by binding of antibody to any part of the chimeric molecule, remain as alternative explanations. However, incubation with the NH_2-terminal anti-LPL antibody had no effect on either the triolein- or tributyrin-hydrolyzing activities of the lipases. Thus, steric hindrance may not play a significant role in the observed antibody inhibition of lipase triolein-hydrolyzing activity. Most importantly, since the incubation of all three lipases with the different anti-HL and LPL antibodies had no significant effect on tributyrin hydrolysis, it is unlikely that major alterations in lipase structure, especially in the catalytic domain, resulted from this interaction.

Analysis of the heparin binding properties of the LPL-chimera by heparin-Sepharose affinity chromatography established its peak elution at 0.9 M NaCl. Therefore, the affinity of the LPL-chimera for heparin was intermediate between that of hLPL and hHL. This finding differs from the results of Davis et al. (16) who reported that the peak elution of their LPL-HL chimeric lipase was identical to that of rat HL. The difference in heparin affinity of the two chimeras may be, in part, due to a 27% difference between the primary amino acid sequence of the C-terminal domain of rat HL (residues 330–472) and that of human HL (residues 330–476). In fact, the human HL C-terminal domain contains 22 basic residues whereas the corresponding rat HL-terminus has only 16 basic amino acids. Additionally, there are two potential N-glycosylation sites in the C-terminal domain of hHL, and only one in the rat HL C-terminus. The number and type of glycosylated residues may, by their size and charge, indirectly contribute to the overall heparin binding properties of the lipases. We have identified two potential heparin binding regions in hLPL (260 to 267 and 441 to 446) which are enriched in basic amino acid residues while devoid of acidic residues, in addition to the region of the consensus sequence KVRAKRSSK which we have extended to include residues (292–306). We hypothesize that all three regions may be required to maintain heparin affinity. Two of the sites (260–267 and 292–306) are located within the proteolytic cleavage fragment of LPL previously demonstrated to bind [^{35}S]heparin (18). The third site (441 to 446) is absent in the LPL-chimera and could potentially account for the chimera's lower heparin affinity relative to that of LPL. Alternatively, the three-dimensional structure assumed by the LPL-chimera may alter its interaction with heparin.

In summary, we have expressed and characterized a human chimeric lipase consisting of the 314 amino-terminal residues of hLPL and the 146 carboxyl-terminal residues of hHL. We conclude that the site of interaction between LPL and its cofactor, apoC-II, resides in the first 314 amino acids of hLPL. The LPL-chimera heparin binding properties were intermediate between those of LPL and HL indicating that both NH_2- and C-terminal regions contribute to heparin binding. Inhibition studies using specific antibodies to HL and LPL indicate that the carboxyl-terminal domain plays an important role in lipid substrate interaction. Analysis of the LPL-chimera has provided new insights into three important functional domains of LPL.

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