Accelerated Publications

Functional Topology of a Surface Loop Shielding the Catalytic Center in Lipoprotein Lipase†

Fabrizia Faustinella, Louis C. Smith, and Lawrence Chan*

Departments of Cell Biology and Medicine, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030

Received April 30, 1992

ABSTRACT: Lipoprotein lipase (LPL), hepatic lipase, and pancreatic lipase show high sequence homology to one another. The crystal structure of pancreatic lipase suggests that it contains a trypsin-like Asp-His-Ser catalytic triad at the active center, which is shielded by a disulfide bridge-bounded surface loop that must be repositioned before the substrate can gain access to the catalytic residues. By sequence alignment, the homologous catalytic triad in LPL corresponds to Asp^{156}-His^{241}-Ser^{132}, absolutely conserved residues, and the homologous surface loop to residues 217–238, a poorly conserved region. To verify these assignments, we expressed in vitro wild-type LPL and mutant LPLs having single amino acid mutations involving residue Asp^{156} (to His, Ser, Asn, Ala, Glu, or Gly), His^{241} (to Asn, Ala, Arg, Gln, or Trp), or Ser^{132} (to Gly, Ala, Thu, or Asp) individually. All 15 mutant LPLs were totally devoid of enzyme activity, while wild-type LPL and other mutant LPLs containing substitutions in other positions were fully active. We further replaced the 22-residue LPL loop which shields the catalytic center either partially (replacing 6 of 22 residues) or completely with the corresponding hepatic lipase loop. The partial loop-replacement chimeric LPL was found to be fully active, and the complete loop-replacement mutant had ~60% activity, although the primary sequence of the hepatic lipase loop is quite different. In contrast, replacement with the pancreatic lipase loop completely inactivated the enzyme. Our results are consistent with Asp^{156}-His^{241}-Ser^{132} being the catalytic triad in lipoprotein lipase. Furthermore, they indicate that the surface loop that shields the active center in the absence of substrate does not have an absolute sequence requirement; replacement of the loop with a different sequence (such as hepatic lipase) having similar predicted secondary structures appears capable of appropriate movement to allow water-insoluble substrates to reach the catalytic center of LPL for hydrolysis.

Lipoprotein lipase (EC 3.1.1.34) (LPL)[1] is an essential enzyme in lipoprotein metabolism. It hydrolyzes the triglyceride component of circulating chylomicrons and very low density lipoproteins (Cryer, 1981; Garfinkel & Schotz, 1987; Robinson, 1987). The enzyme shows high sequence homology to hepatic triglyceride lipase and pancreatic lipase (Ben-Zeev et al., 1987; Datta et al., 1988). Interest in the enzymology of triglyceride hydrolysis has been greatly stimulated by the 1990 reports of the 1.9-Å structure of *Rhizomucor miehei* lipase (Brady et al., 1990) and the 2.4-Å structure of human pancreatic lipase (Winkler et al., 1990), which show that the catalytic center of the two lipases contains a serine protease-like catalytic triad of Ser···His···Asp. Other features in common for these α/β proteins include a central core of a mixed β-sheet containing the catalytic triad and a surface loop restricting the access of the substrate to the active site. The 2.2-Å structure of a third lipase, from *Geotrichum candidum* (Schrag et al., 1991), has the same general structural features, including the surface loop, but differs in that Glu replaces Asp in the catalytic triad. The concensus structure of the lipases involves (a) the catalytic triad and its topology, (b) a central core of a mixed β-sheet containing the catalytic triad, and (c) a surface loop restricting the access of the substrate to the active site. The crystal structure of pancreatic lipase also contains a loop or flap which appears to shield the active site from the substrate (Winkler et al., 1990). By sequence alignment, LPL and hepatic triglyceride lipase also contain an analogous loop bounded by two conserved Cys residues. The amino acid sequence of the loop is poorly conserved compared to the rest of the lipase molecule (Datta et al., 1988). Here we demonstrate that multiple single amino acid substitution mutants involving Asp^{156}, His^{241}, or Ser^{132} individually are all inactive, indicating that they represent the catalytic triad in LPL. Furthermore, partial and complete replacement of the LPL loop with the hepatic triglyceride lipase loop, but not the pancreatic lipase loop, produces an active LPL enzyme. Thus, the presumed reorientation of this loop which must take place before LPL can hydrolyze water-insoluble substrates at water–lipid interfaces does not have an absolute sequence requirement. A functional topology is achieved by the considerable compositional flexibility allowed in the primary sequence of the loop.

MATERIALS AND METHODS

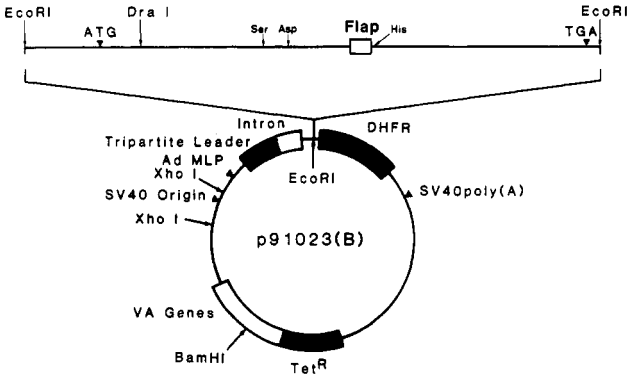
Human LPL cDNA Expression Vector. A 1786-bp human LPL cDNA containing the entire coding region bounded by an artificial *Hin*dIII site in the 5′ end and a natural *Eco*RI site in the 3′ end is subcloned into M13Mp19 and used as a

† This work was supported by National Heart, Lung, and Blood Institute Grants HL16512 (to L.C.) and HL27341 for a Specialized Center of Research in Arteriosclerosis (to L.C.S.) at Baylor College of Medicine and by a grant from the March of Dimes Birth Defects Foundation to L.C.

* Correspondence should be addressed to this author at the Department of Cell Biology.

[1] Abbreviation: LPL, lipoprotein lipase.

0006-2960/92/0431-7219$03.00/0



LPL loop replacement mutants

Lipoprotein lipase (wild-type):	(wt)	NIGEAIRVIAERGLGDVDQLVK
LPL-hepatic lipase (partial):	(LPL-pH)	HSLELYRHIAERGLGDVDQLVK
LPL-hepatic lipase (complete):	(LPL-cH)	HSLELYRHIAQHGFNAITQTIK
LPL-pancreatic lipase (partial):	(LPL-pP)	KKNALSQIIAERGLWEGTRDFVA
LPL-pancreatic lipase (complete):	(LPL-cP)	KKNALSQIVNLDGIWEGTRDFVA

LPL loop deletion mutants

D-5	NIGEAI-VIA-RG-GD-DQ-VK
D-11	NI-EA--V-A--G-G--D--VK

FIGURE 1: (A, top) Lipoprotein lipase expression vector. Residues Asp[156], His[241], and Ser[132] are indicated. Multiple single amino acid substitution mutants were constructed to replace each of these residues in turn. The region marked Flap encompasses the sequence encoding a 22-residue peptide, Asn[217]–Lys[238], bounded by two cysteine residues, referred to as the loop region in the text. (B, bottom) Structure of the flap or loop region. The amino acid sequence for each loop is shown in single-letter code. The mutant sequence is underlined. The abbreviations are as defined in the figure. For the deletion mutants, the deleted amino acids are denoted by dashes. The oligonucleotides used for creating the chimeric mutants are as follows: for LPL-pH, CCAGGATGTCACTTTCTAGAACTTTACCGCCATATTGCAGAG; for LPL-cH, CCAGGATGTCACTTTCTAGAACTTTACCGCCATATTGCACAGCACGGATTTAATGCTATCACCCAGACAATAAAGTGCTCCCAC; for LPL-pP, CAGCCAGGATGTAAGAAGAATGCACTTAGCCAGATCATTGCAGAG and AGAGGACTTTGGGAAGGGACCCGGGACTTCGTGGCTTGCTCCCACGAG; for LPL-cP, an additional oligonucleotide, ACCCAGATCGTTGACCTGGATGGAATTTGGGAAGGG, was used to complete the loop replacement on LPL-pP. For the deletion mutants, the following oligonucleotides were used: for D-5, GGAGAAGCTATCGTGATTGCAAGAGGAGGAGATGACCAGGTGAAGTGC; for D-11, GGATGTAACATTGAAGCTGTGGCAGGAGGAGACGTGAAGTGCTCCCAC. These oligonucleotides were synthesized on an Applied Biosystems Model 391 DNA synthesizer. All mutant clones were confirmed by direct DNA sequencing. For both LPL and hepatic triglyceride lipase, the human sequences were used. For the pancreatic lipase loop, the dog sequence (Mickel et al., 1989) was used because, by Chou–Fasman analysis, its structure is more similar to the LPL loop than is the human pancreatic lipase sequence, which differs from the dog sequence by four conservative amino acid substitutions.

template for site-directed mutagenesis (Semenkovich et al., 1990) (Figure 1A).

Oligonucleotides were synthesized on an Applied Biosystems Inc. 391 DNA synthesizer. The sequences of the oligonucleotides used to introduce mutations in the catalytic triad residues are as follows: All oligonucleotides correspond to the sense strand. They are 27 nucleotides in length and contain 12 bases on either side of the mutated codon. The sequences of the mutated codons are Asp[156] (→ His, CAT; Ser, AGT; Asn, AAT; Ala, GCT; Gla, GAA; Gly, GGT), His[241] (→ Asn, AAC; Ala, GCC; Arg, CGC; Gln, CAG; Trp, TGG), and Ser[132] (→ Gly, GGC; Ala, GCC; Thr, ACC; Asp, GAC). The sequences for the oligonucleotides used for mutating the loop segment are listed in the legend to Figure 1B. The oligonucleotides were 5′-phosphorylated and annealed with single-stranded template. Mutagenesis was carried out as described by Taylor et al. (1985) using an oligonucleotide-directed in vitro mutagenesis kit (Amersham Corp.). Mutant and wild-type LPL cDNAs were used to transfect *Escherichia coli* TG1 cells, and positive clones were identified by direct sequencing. Replicative-form DNAs were isolated, digested with *Eco*RI, and inserted into the *Eco*RI site of p91023(B) (Wong et al., 1985). After transformation of *E. coli* DH 5α cells, positive clones were isolated, and orientation of inserts was checked by restriction mapping.

In Vitro Expression and LPL Enzyme Assay. COS M-6 cells [(0.25–1.0) × 10^6] were plated on 100-mm-diameter tissue culture dishes and transfected with 20 μg of plasmid DNA/dish by the technique of Selden et al. (1986) with the addition of a 3-h incubation with chloroquine after Me_2SO shock. In all experiments, collecting media contained sodium heparin (40 μg/mL). In each experiment, a parallel dish was subjected to the transfection protocol without plasmid DNA (COS cells only condition). For LPL enzyme assay experiments, after 48–72 h, aliquots of culture media were collected. Cells were washed in phosphate-buffered saline, scraped into 1 mL of 50 mM NH_3/NH_4Cl (pH 8.1) containing heparin, and sonicated. Media and cell extracts were then flash-frozen in a dry ice/ethanol bath and stored at −70 °C. Just before determination of LPL activity or protein mass, samples were thawed in ice water, made 0.2% in sodium deoxycholate, and rotated at room temperature for 5 min. Cell extracts and media were assayed for LPL enzyme activity as previously described (Semenkovich et al., 1990). LPL activity is expressed in milliunits (1 milliunit = 1 nmol of fatty acid released per minute). In experiments determining enhancement of LPL activity of apolipoprotein C-II, media from transfected COS cells were assayed in the presence of solvent (3 M guanidine hydrochloride diluted to a final concentration of 12 mM) or of purified human apolipoprotein C-II (kindly provided by Dr. Henry Pownall, Baylor College of Medicine) in the same solvent. As a control, bovine milk LPL was purified as previously described (Voyta et al., 1985) and assayed under the same conditions.

Quantitation of LPL Protein Mass. LPL mass was determined by a standard enzyme-linked immunosorbent assay using purified bovine milk LPL as standard. The assay utilized a monoclonal antibody raised against purified bovine milk LPL (monoclonal antibody 40) as described by Voyta et al. (1985).

RESULTS AND DISCUSSION

The results of the in vitro expression experiments are presented in Table I. For the wild-type construct, about 70% of the immunoreactive LPL is present in the culture media and 30% in the cellular extract. Enzyme activity is also readily detected in both compartments. The specific activities of wild-type LPL expressed in vitro are comparable to that of LPL purified from bovine milk (data not shown). The wild-type LPL also shows a 5-fold stimulation of activity with the addition of purified apolipoprotein C-II, a known physiological activator of LPL (LaRosa et al., 1970). All assays shown in Table IB,C were performed in the presence of apolipoprotein C-II. Untransfected COS cells or cells transfected with the expression vector without the LPL cDNA insert do not produce any detectable LPL.

A large number of single amino acid substitutions have been reported that result in an inactive LPL [summarized by Hayden et al. (1991)]. However, when multiple substitutions

Table I: Lipoprotein Lipase Activity in Wild-Type LPL and Site-Specific Mutants Produced in COS Cells[a]

(A) Lipoprotein Lipase

	ApoC-II (725 nM)	LPL activity (mean ± SD, milliunits/dish) cell	media
vector without insert	+	0	0
wild-type LPL	-	103 ± 8.76	370 ± 26.1
	+	566.7 ± 41.3	1700.2 ± 125.5

(B) Catalytic Residue Mutants[b]

	LPL activity (milliunits/dish) cell	media	LPL mass (μg/dish) cell	media	specific activity (milliunits/μg) cell	media
wild-type	620 ± 50.2	1820 ± 110	18 ± 1.5	56 ± 2.5	34	32
Asp^{156} → His	0	0	11 ± 2.5	31 ± 4	0	0
Ser	0	0	10 ± 0.9	30 ± 2.4	0	0
Asn	0	0	9 ± 1.7	28 ± 3	0	0
Ala	0	0	11 ± 3	32 ± 3.5	0	0
Glu	0	0	12 ± 2.5	30 ± 3.4	0	0
Gly	0	0	6 ± 1.2	25 ± 2	0	0
His^{241} → Asn	0	0	10 ± 1.8	29 ± 2.5	0	0
Ala	0	0	12 ± 3	31 ± 4	0	0
Arg	0	0	12 ± 3.2	32 ± 4.2	0	0
Gln	0	0	11 ± 2.7	28 ± 2.9	0	0
Trp	0	0	10 ± 1.9	28 ± 2.5	0	0
Ser^{132} → Gly	0	0	15 ± 2	45 ± 3	0	0
Ala	0	0	12 ± 1.6	40 ± 3	0	0
Thr	0	0	8 ± 0.8	35 ± 1.8	0	0
Asp	0	0	10 ± 0.9	46 ± 2.4	0	0

(C) Loop Segment Mutants

	LPL activity (milliunits/dish) cell	media	LPL mass (μg/dish) cell	media	specific activity (milliunits/μg) cell	media
wild-type	530 ± 41.5	1750 ± 130	17 ± 2.1	50 ± 3.5	31	35
LPL-pH	500 ± 40	1700 ± 131	16 ± 1.9	48 ± 3	31	35
LPL-cH	310 ± 38	1050 ± 100	16 ± 2.5	47 ± 2.8	19	22
LPL-pP	0	0	6.5 ± 1.9	21 ± 1.8	0	0
LPL-cP	0	0	7 ± 1.7	20 ± 2	0	0
LPL-D-5	0	0	11 ± 2.5	26 ± 2.3	0	0
LPL-D-11	0	0	6 ± 0.8	21 ± 0.9	0	0

[a] The data represent the results from one set of experiments in which triplicate transfections were performed. These experiments were repeated at least three times with consistent results. The abbreviations for the loop-replacement and deletion mutant LPLs are as described in Figure 1. [b] Seven additional positive controls using other LPL mutants (described in the text) analyzed simultaneously had activities similar to that of wild type. The results of these are not included here because they have been published previously (Semenkovich et al., 1990; Faustinella et al., 1991a,b).

are made in some of these sites, some of the mutants have LPL activity (Faustinella et al., 1991a). Therefore, it is dangerous to draw conclusions from studies on one or two mutations at each site. We have therefore tested a total of 15 single amino acid substitution mutant LPLs, 6 for Asp^{156}, 5 for His^{241}, and 4 for Ser^{132}. Taken together, previous studies using site-directed mutagenesis of the conserved active site residues of serine proteases and related enzymes (Walsh, 1979) show that there is an almost absolute requirement for the Ser, Asp, and His in the catalytic triad. The results of our study reinforce this conclusion. While we anticipated any substitution should give an inactive protein, we used the following rationale for the selection of the triad mutants. For Ser^{132}, substitution by both Gly and Ala would create minimal disturbance of the overall structure while precluding the formation of an acyl intermediate. The formation of an acyl intermediate is theoretically possible for the Thr and Asp mutants. Except for the standard Gly and Ala substitutions, the other mutations at Asp^{156} involve amino acid side chains with hydrogen-bonding capability: Gln, Asn, His, and Ser. The selection of Asn, Arg, Gln, and Trp for His^{241} was based on the same hydrogen donor–acceptor potential. As expected, all LPL mutants were found to be totally inactive (Table IB). The wild-type LPL and seven other mutant LPLs assayed in the same experiment showed good activity. These enzymatically active mutants, which had been published previously (Semenkovich et al., 1990; Faustinella et al., 1991a,b), include the following: Ser^{45} → Thu, Ser^{143} → Ala, Ser^{244} → Ala, Asn^{257} → Ala, Asn^{359} → Ala, Ser^{363} → Thu, and Gly^{448} → Ter. Our current study extends the previous observation on a single mutant involving Asp^{156} (Faustinella et al., 1991b) and three mutants involving Ser^{132} (Faustinella et al., 1991a) that these residues may be important for enzyme catalysis. Taken together, the complete loss of the enzyme activity in the 15 LPL mutants involving Asp^{156}, His^{241}, or Ser^{132} supports the conclusion that these three residues constitute the catalytic triad in LPL, as suggested by the sequence alignment against pancreatic lipase.

The crystal structure of human pancreatic lipase predicts that Asp^{176}-His^{263}-Ser^{152} form a catalytic triad in the active center of the enzyme. By sequence alignment between pancreatic lipase and LPL, Asp^{156}-His^{241}-Ser^{132} constitute the catalytic triad residues in LPL. In order to obtain an understanding of the structural basis of LPL action and to obtain direct experimental proof for the essential nature of these residues, we have expressed LPL in vitro and have examined the enzymatic activities of the wild-type enzyme and site-specific single amino acid substitution mutants involving the putative catalytic triad residues. After these studies had been completed, a report by Emmerich et al. (1992) appeared which corroborates the conclusions reached by our studies.

The X-ray structure of pancreatic lipase suggests that a substantial conformational change occurs in the enzyme molecule before it can bind substrate in the active center defined by the catalytic triad residues. There is a surface loop between disulfide-bridged residues (residues 237–261) that covers the active site. This loop has to be repositioned before the site can become accessible to substrate. The loop segments which shield the catalytic center of pancreatic lipase, LPL, and hepatic triglyceride lipase are easily identified by sequence alignment because they are bounded on both sides by cysteine residues that are, to date, absolutely conserved in all three enzymes across all species. In pancreatic lipase (Winkler et al., 1990) and LPL (Yang et al., 1989), these two cysteine residues have been shown to exist in disulfide linkage. The expectation that the surface loop of lipase must move before the substrate can diffuse into the catalytically active site has been confirmed in an elegant study by Brzozowski et al. (1991). A lipase–inhibitor conjugate was prepared by the stiochiometric reaction of *n*-hexyl chlorophosphonate ethyl ester with the *R. miehei* lipase, which inhibited it fully and irreversibly. The structure of the crystals of the covalently modified enzyme demonstrated that the 15 amino acid "lid" undergoes a hinge-type motion that transports some of the atoms of a short α-helix (residues 85–91) over a distance of 12 Å. Movement of the helical lid changes the hydrophobic–hydrophilic balance of the exterior surface of the lipase. The hydrophilic side of the lid, which is exposed as a solvent in the native structure, is partially buried in the polar cavity previously filled with water. At the same time, the hydrophobic side of the lid becomes completely exposed, greatly expanding the nonpolar surface around the active site. The analysis of conformational differences between the native and inhibited enzymes shows conclusively that the conformational changes are essential for catalytic activity of the enzyme. Moreover, the structural details of the conformation of the inhibited lipase strongly support the view that the observed changes are characteristic of the activated enzyme adsorbed at the oil–water interface.

In the first set of experiments on the LPL loop, we replaced it either partially (replacing 6 of 22 residues) or completely with the corresponding loop from hepatic lipase (Figure 1). The partial-replacement chimeric LPL expressed in vitro was fully active, with a specific activity identical to that of the wild-type enzyme. The complete-replacement chimeric molecule was also active, with a specific activity approximately 60% that of the wild-type enzyme (Table IC). In the next series of experiments, we replaced the LPL loop with the pancreatic lipase loop, either partially (replacing 6 of 22 residues) or completely (replacing the 22 LPL loop residues with a 23-residue pancreatic lipase loop) (Figure 1). Both types of chimeric LPL were totally inactive, although immunoreactive protein was synthesized and secreted. We next deleted 5 and 11 residues from the LPL loop. Both LPLs with shortened loop regions were produced by the COS cells, but they were totally devoid of enzyme activity (Table IC).

The study summarized in Table IC addresses a specific structure–function relationship in LPL: the loop region of LPL that appears to prevent the substrate from reaching the active center. Replacement of this portion of the enzyme structure with the corresponding regions of either hepatic triglyceride lipase or pancreatic lipase appeared to be a reasonable choice for site-specific mutagenesis for the following reasons. (a) This portion of the lipases is isolated from the main structural domains of the proteins by a disulfide bridge. (b) There is little conservation of the primary sequence, although, as noted below, predictions for the structure of this region of LPL and hepatic triglyceride lipase are similar. (c) We reasoned that the loop regions of LPL and hepatic triglyceride lipase should have a high degree of functional similarity since the physiological substrates for both enzymes are plasma lipoproteins (Jackson, 1983). LPL utilizes as substrates almost exclusively the large triglyceride-rich chylomicrons and very low density lipoproteins. Hepatic triglyceride lipase acts on a much more diverse group of lipoprotein particles, with significant hydrolysis of both phospholipids and triglycerides in very low density, intermediate density, and high density lipoproteins. The diameters of these macromolecular substrates range from 9–12 nm for high density lipoprotein-2 to 1200 nm for chylomicrons. By contrast, the bile salt micellar substrates for pancreatic lipase have diameters of ~7 nm. Thus, from the perspective of the lipid–water interface of the respective substrates, the repositioning of the loop region of these lipases and their possible role in substrate binding would be similar for LPL and hepatic triglyceride lipase on one hand but quite different for pancreatic lipase on the other hand.

Our previous studies using site-directed mutagenesis of LPL (Faustinella et al., 1991a), as well as analysis of naturally occurring human mutations (Hayden et al., 1991; Faustinella et al., 1991b), give strong support to the view that the secondary and tertiary structures of human pancreatic lipase and LPL are quite similar (Derewenda & Cambillau, 1991). Several computational approaches are available for possible secondary structures derived by analysis of the primary amino acid sequence of protein (Benner & Gerloff, 1990; Garnier et al., 1978). An algorithm based on the Chou–Fasman prediction rules (Chou & Fasman, 1978) gave a predicted structure of the loop region of human pancreatic lipase that was in reasonable agreement with the secondary structure of the pancreatic lipase loop determined by X-ray crystallography (Winkler et al., 1990; Derewenda & Cambillau, 1991). Our analysis of the LPL expression studies are based on the results of the Chou–Fasman predictions.

For the LPL loop, there is high probability for an α-helical structure for Ile^{218}–Glu^{220} and a β-sheet for Ala^{221}–Arg^{223}, followed by another α-helical region Val^{224}–Gly^{229}. The remainder of the loop, Leu^{230}–Lys^{238}, is predicted to be a β-sheet, interrupted by a highly probable β-turn at Asp^{232}. The predicted structure of the first α-helical region of the hepatic triglyceride lipase loop, His-Ser-Leu-Glu-Leu-Tyr (residues 217–222), is the same as that predicted for the LPL loop, Asn-Ile-Gly-Glu-Ala-Ile (residues 217–222). This assignment is fully consistent with the experimental finding that partial replacement of the hepatic triglyceride lipase sequences produces a mutant LPL with full lipolytic activity. In the mutant LPL containing the entire loop region of hepatic triglyceride lipase, the primary sequence of the latter is quite different from the LPL loop. The high level of activity of this complete-replacement mutant is significant in light of the absence of enzyme activity in many single amino acid substitution mutants tested in the past (Semenkovich et al., 1990; Hayden et al., 1991; Faustinella et al., 1991a,b). The Chou–Fasman algorithm predicts, however, the same alternating pattern of α-helical and β-sheet regions involving approximately the same number of residues in the LPL and the hepatic triglyceride lipase loop. The Asp^{234} to Ala exchange in the mutant negates the β-turn probability in the terminal β-sheet portion of the loop of LPL with only minimal reduction in activity. The predicted structures for the mutants containing the partial and complete loop sequence from the pancreatic lipase are also similar to that of LPL, but these

mutants are totally inactive. One possibility is that replacement of Arg^{228} by Asp in the loop region changes the polarity of the extended loop so that any substrate-induced conformation stabilized through ionic interaction between different sequence regions of the LPL would not be possible. The inactive partial deletion LPL mutants are not informative, except that, in these mutants, significant changes in secondary structure are predicted; the loop may also require a certain length for appropriate movement and repositioning.

Using multiple single amino acid substitution mutants of LPL, we have demonstrated that residues Asp^{156}, His^{241}, and Ser^{132} are essential for LPL activity. This observation, coupled with the sequence alignment against the putative catalytic triad residues in pancreatic lipase, supports the conclusion that Asp^{156}-His^{241}-Ser^{132} indeed are the catalytic triad residues in the active center of LPL (Emmerich et al., 1992). Furthermore, we have demonstrated that the loop shielding the active center in LPL can be replaced with the corresponding structure from hepatic triglyceride lipase without significant loss of enzyme activity. Substitution with a pancreatic lipase loop inactivates the enzyme. The different results with the two types of loop replacement may be related to the substantial difference in size of the physiological substrate presented to LPL and hepatic triglyceride lipase on the one hand and pancreatic lipase on the other. Although the invertebrate and vertebrate lipases have entirely different primary structures, they exhibit great similarity in the fold structure and considerable analogy in the topology of the catalytic residues and the presence of this helical loop which must be repositioned before the active site becomes accessible to the substrate [reviewed by Smith et al. (1992)]. Our study documents the interchangeability of the LPL and hepatic triglyceride lipase loop, strongly suggesting a common functional topology of this loop in these two lipases.

REFERENCES

Benner, S. A., & Gerloff, D. (1990) *Adv. Enzyme Regul. 31*, 121–181.

Ben-Zeev, O., Ben-Avram, C. M., Wong, H., Nikazy, J., Shively, J. E., & Schotz, M. C. (1987) *Biochim. Biophys. Acta 919*, 13–20.

Brady, L., Brzozowski, A. M., Derewenda, Z. S., Dodson, E., Dodson, G., Tolley, S., Turkenburg, J. P., Christiansen, L., Hughe-Gensen, B., Norskov, L., Thim, L., & Menge, U. (1990) *Nature 343*, 767–770.

Brzozowski, A. M., Derewenda, U., Derewenda, Z. S., Dodson, G. G., Lawson, D. M., Turkenburg, J. P., Bjorkling, F., Hughe-Gensen, B., Patkar, S. A., & Thim, L. (1991) *Nature 351*, 491–494.

Chou, P. Y., & Fasman, G. D. (1978) *Annu. Rev. Biochem. 47*, 251–276.

Cryer, A. (1981) *Int. J. Biochem. 13*, 525–541.

Datta, S., Luo, C.-C., Li, W.-H., Van Tuinen, P., Ledbetter, D. H., Brown, M. A., Chen, S.-H., Liu, S.-W., & Chan, L. (1988) *J. Biol. Chem. 263*, 1107–1110.

Derewenda, Z. S., & Cambillau, C. (1991) *J. Biol. Chem. 266*, 23112–23119.

Emmerich, J., Beg, O. U., Peterson, J., Previato, L., Brunzell, J. D., Brewer, H. B., Jr., & Santamarina-Fojo, S. (1992) *J. Biol. Chem. 267*, 4161–4165.

Faustinella, F., Chang, A., Van Biervliet, V. P., Rosseneu, M., Vinaimont, N., Smith, L. C., Chen., S.-H., & Chan, L. (1991a) *J. Biol. Chem. 266*, 14418–14424.

Faustinella, F., Smith, L. C., Semenkovich, C. F., & Chan, L. (1991b) *J. Biol. Chem. 266*, 9481–9485.

Garfinkel, A. S., & Schotz, M. C. (1987) in *Plasma Lipoproteins* (Gotto, A. M., Jr., Ed.) pp 335–357, Elsevier Scientific Publishing Co., New York.

Garnier, J., Ogusthorpe, D. J., & Robson, B. (1978) *J. Mol. Biol. 120*, 97–120.

Hayden, M. R., Ma, Y., Brunzell, J., & Henderson, H. E. (1991) *Curr. Opin. Lipidol. 2*, 104–109.

Jackson, R. L. (1983) in *The Enzymes* (Boyer, P. D., Ed.) Vol. 16, pp 141–181, Academic Press, Orlando, FL.

LaRosa, J. C., Levy, R. I., Herbert, P., Lux, S. Z., & Frederickson, D. S. (1970) *Biochem. Biophys. Res. Commun. 41*, 57–62.

Mickel, F. S., Weidenbach, F., Swarovsky, B., LaForge, K. S., & Scheele, G. A. (1989) *J. Biol. Chem. 264*, 12895–12901.

Robinson, D. S. (1987) in *Lipoprotein Lipase* (Brensztajn, J., Ed.) pp 1–13, Evener Publishing, Inc., Chicago, IL.

Schrag, J. D., Li, Y., Wu, S., & Cygler, M. (1991) *Nature 351*, 761–764.

Selden, R. F., Howie, K. B., Rowe, M. E., Goodman, H. M., & Moore, D. D. (1986) *Mol. Cell. Biol. 6*, 3173–3179.

Semenkovich, C. F., Luo, C. C., Nakanishi, M. K., Chen, S.-H., Smith, L. C., & Chan, L. (1990) *J. Biol. Chem. 265*, 5429–5433.

Smith, L. C., Faustinella, F., & Chan, L. (1992) *Curr. Opin. Struct. Biol.* (in press).

Taylor, J. W., Ott, J., & Eckstein, F. (1985) *Nucleic Acids Res. 13*, 8765–8785.

Voyta, J. C., Via, D. P., Kinnunen, P. K. J., Sparrow, J. T., Gotto, A. M., Jr., & Smith, L. C. (1985) *J. Biol. Chem. 260*, 893–898.

Walsh, C. (1979) *Enzymatic Reaction Mechanisms*, pp 94–107, W. H. Freeman & Co., New York.

Winkler, F. K., D'Arcy, A., & Hunziker, W. (1990) *Nature 343*, 771–774.

Wong, G. G., Witek, J. S., Temple, P. A., Wilkens, K. M., Leary, A. C., Luxenberg, D. P., Jones, S. S., Brown, E. L., Kay, R. M., Orr, E. C., Shoemaker, C., Golde, D. W., Kaufman, R. J., Hewick, R. M., Wang, E. A., & Clark, S. C. (1985) *Science 228*, 810–815.

Yang, C.-Y., Gu, Z.-W., Yang, H.-X., Rohde, M. F., Gotto, A. M., Jr., & Pownall, H. J. (1989) *J. Biol. Chem. 264*, 16822–16827.