Biochemistry

 Volume 28, Number 10 May 16, 1989

Accelerated Publications

Structure of the Human Lipoprotein Lipase Gene†

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Received January 30, 1989; Revised Manuscript Received March 2, 1989

ABSTRACT: Human genomic clones that span the entire lipoprotein lipase (LPL) gene have been isolated and used to determine its structure. The gene is approximately 30 kilobase (kb) pairs in length in which the mRNA specifying sequence is divided into 10 exons. Exons 1–9 are of average size (105–276 bp) whereas exon 10, which specifies the entire 3′ uncoding sequence, is 1948 bp in length. Exon 1 codes for the signal peptide, exon 2 includes the protein domain that was shown to bind to the lipoprotein substrate, and exons 6 and 9 code for sequences that are relatively rich in basic amino acids and therefore likely to be involved in anchoring of the enzyme to the capillary endothelium by interaction with the acidic domain of heparan sulfate. Four closely spaced mRNA 5′ termini were observed, indicating multiple transcription initiation sites, one of which seems to be favored. Two potential enhancer sequence motifs in the 5′ upstream region were observed. One may specify expression in response to intracellular Ca^{2+} mobilization, and the other may be responsible for expression in adipocytes.

Lipoprotein lipase (LPL; triacylglycero-protein acylhydrolase, EC 3.1.1.34) catalyzes the hydrolysis of core triacylglycerols of circulating very low density lipoproteins (VLDL) and chylomicrons generating fatty acids for storage in adipose tissue or oxidation in muscle. This enzyme therefore plays a crucial role in plasma lipoprotein processing and energy metabolism in general (Eckel, 1987). LPL is a glycoprotein synthesized and secreted by a variety of parenchymal cells, including adipocytes, skeletal and cardiac muscle cells, and macrophages (Nilsson-Ehle et al., 1980; Mahoney et al., 1982; Chait et al., 1982). After secretion it becomes bound to glycosaminoglycans on the luminal surface of capillaries. Apolipoprotein CII, which is present on the surface of VLDL and chylomicrons, was shown to act as a cofactor for LPL (Olivecrona & Bengtsson-Olivecrona, 1987).

Human, bovine, and mouse cDNA clones of LPL have recently been isolated (Wion et al., 1987; Auwerx et al., 1988; Senda et al., 1987; Kirchgessner et al., 1987). The human LPL gene has been assigned to 8p22 (Sparkes et al., 1987). The deduced amino acid sequence is extraordinarily conserved among these species. The sequence of LPL is also homologous to those of hepatic lipase and pancreatic lipase, indicating that they may belong to a family of lipases (Ben-Avram et al., 1986; Komaromy et al., 1987). In the present study we describe the isolation of human genomic clones that have allowed elucidation of the entire structure of the LPL gene.

Materials and Methods

Isolation of LPL Genomic Clones. Four overlapping genomic clones averaging 35 kb in length encompassing the entire LPL gene and approximately 25 kb of the flanking regions were isolated from a human cosmid library (kindly provided by G. McKnight, Zymogenetics, Inc., Seattle, WA). The library was screened with a ^{32}P-labeled (random primed labeling kit supplied by Boehringer Mannheim, Indianapolis, IN; specific activity of approximately 1×10^9 cpm/μg) human cDNA probe isolated in our laboratory [HLPL 26, 1.36 kb in length corresponding to base pairs 271–1630 of the sequence reported by Wion et al. (1987)]. Colony lifts on nitrocellulose membranes were prepared and hybridized to the probe according to the procedure of Wallace et al. (1979) and as modified by Deeb et al. (1985).

Mapping and Sequencing of Genomic Clones. Restriction enzyme mapping was first performed on the cosmid clones with the enzymes *Xba*I, *Sst*I, and *Bam*HI, which had a few recognition sites on these clones. More detailed mapping and sequencing were performed on subclones in the plasmid vectors pGEM-1 and pGEM-3Z*f*(+) (Promega Corp., Madison, WI).

† This work was supported by NIH Grants HL 30086, DK 02456, and GM 15253.

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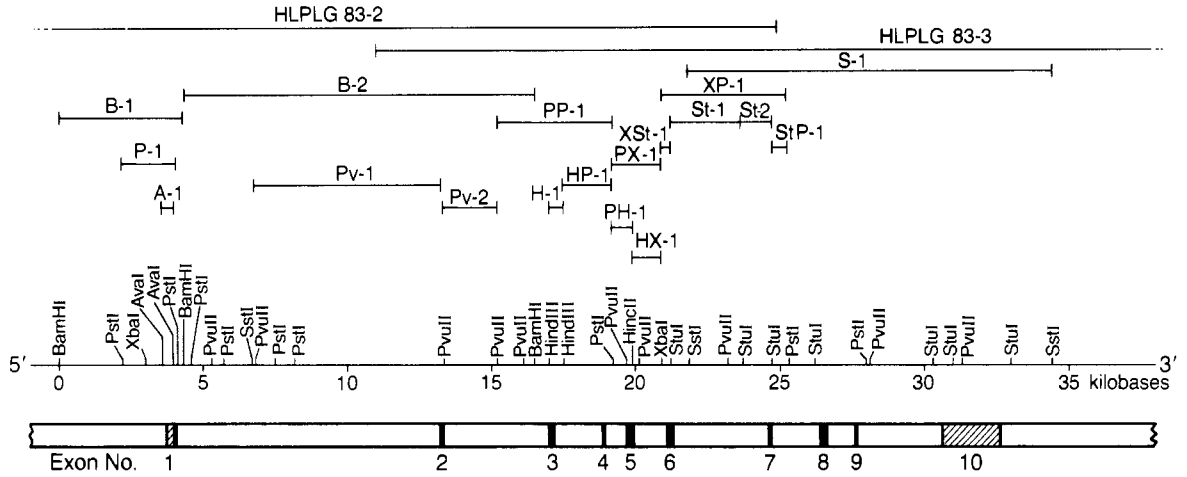

FIGURE 1: Structure of the human LPL gene. The 10 exons are represented by filled boxes. Shaded portions of exons represent noncoding sequences. The two solid lines at the top labeled HLPLG 83-2 and 83-3 represent the two overlapping cosmid clones that contain the whole gene. Solid lines below these cosmid clones represent subclones in pGEM-1 and pGEM-3Z *f*(+) plasmids used in sequencing and mapping. Restriction endonuclease sites are indicated on the scale, which is kilobase pairs. Not all of the *Ava*I, *Hinc*II, *Hin*dIII, and *Stu*I sites have been included. The *Xba*I site in exon 10 overlaps with the palindromic recognition sequence GATC of a specific *E. coli* methylase (*dam*$^-$ gene product). Therefore, *Xba*I cuts at this site only in subclones that have been propagated in *E. coli dam*$^-$ cells.

Sequencing was performed by the dideoxy chain termination method (Sanger et al., 1977) on double-stranded circular DNA templates using T7, SP6, or synthetic oligonucleotides (corresponding to cDNA sequences) as primers. The "sequenase" DNA sequencing kit supplied by the U.S. Biochemical Corp. (Cleveland, OH) was used. All of the coding portion of the gene was sequenced together with intron–exon boundaries. Exons 9 and 10 were initially localized, employing the polymerase chain reaction (PCR) to amplify genomic fragments encompassed by two oligonucleotide primers in adjacent exons (Saiki et al., 1985).

Sequence homology searches were performed by using the program GENEPRO (Riverside Scientific Enterprises, Seattle, WA).

Primer Extension Analysis. The 5′ terminus of the LPL mRNA was determined by the primer extension method (Calzone et al., 1987). The oligonucleotide primer (4–6 ng) 5′-GCAGCTAGAAGTGGGCAGCT-3′, complementary to the sequence +40 to +59 (see Figure 3), was labeled at the 5′ end by phosphorylation with T4 polynucleotide kinase and [γ-^{32}P]ATP and hybridized to 40 μg of poly(A)+ human adipose tissue RNA. Extension of the primer was performed with AMV reverse transcriptase (Life Sciences, Inc., St. Petersburg, FL). The reaction products were separated on an 8% polyacrylamide–7 M urea sequencing gel and subjected to autoradiography. DNA fragments generated by sequencing a genomic DNA clone (A1 in Figure 1) with the same primer were used as size markers. This analysis, which was performed three times, gave the same extension products.

RESULTS

Isolation and Structure of LPL Genomic Clones. Four overlapping genomic clones (two of which are shown in Figure 1) encompassing the entire LPL gene were isolated by screening a human library with cDNA probes. The length of the LPL gene is approximately 30 kb, which is about nine times the length of the cDNA (Figure 1). The cDNA is interrupted by 9 introns giving rise to 10 exons. Exons 1–9 range in length between 105 and 276 bp, while exon 10, which codes for all of the 3′ untranslated region of the mRNA, is 1948 bp in length. The size and position of exons together with the codons and corresponding amino acids interrupted in introns are given in Table I. The size of introns was estimated by restriction enzyme analysis and in some cases (introns 8 and 9) confirmed by amplification of DNA fragments with the polymerase chain reaction using oligonucleotides corresponding to the two flanking exons. All intron–exon splice junctions were sequenced (Table II) and found to conform to consensus sequences (Breathnach & Chambon, 1981; Mount, 1982). The sequence of exons differs from the cDNA sequence reported by Wion et al. (1987) by a single base in the 5′ untranslated region (C in place of G at position +154).

Table I: Positions and Size of Exons in the Human LPL Gene[a]

exon	position in cDNA and size (bp)	codons interrupted	amino acid and position
1	1–276 (276)	C↓AA	Gln^{30}
2	277–437 (161)	ACG↓GTA	Thr^{83}–Val^{84}
3	438–617 (180)	GAG↓GAG	Glu^{143}–Glu^{144}
4	618–728 (111)	ACT↓GGC	Thr^{180}–Gly^{181}
5	729–963 (235)	G↓AT	Asp^{259}
6	964–1206 (243)	G↓TC	Val^{340}
7	1207–1327 (121)	CT↓G	Leu^{380}
8	1328–1510 (183)	AA↓G	Lys^{441}
9	1511–1615 (105)	TG↓A	termination
10	1616–3563 (1948)		

[a] Positions of amino acid residues are relative to the initiation codon.

Sequencing of the *Pst*I 3′ end of subclone XP-1 (Figure 1) revealed the presence of one copy of a sequence that is characteristic of Alu family members (Schmid & Jelinek, 1982). This repeat starts downstream of the *Pst*I site and ends with an A-rich sequence $(A)_{24}$ 117 bp upstream.

The 5′ Flanking Region of the LPL Gene. The 5′ termini of the adipose tissue LPL mRNA were mapped by primer extension as described under Materials and Methods. Extension product sizes of 55, 56, 59, and 60 bp were observed, indicating that four transcription initiation sites are used at the LPL promoter (Figure 2). The most frequently used initiation site appears to be 188 bp 5′ upstream from the ATG initiation codon and is arbitrarily designated +1 (Figure 3). As primer extension is subject to premature termination, an independent method, such as S1 nuclease protection, would be necessary to confirm the above observation.

The TATA (CATAA) and CCAAT box sequences, which occur at 27 and 65 bp upstream from the major initiation site, are indicated with lines above and below (Figure 3). The following potential enhancer sequence motifs are also present in the 5′ upstream region: the octanucleotide sequence 5′-

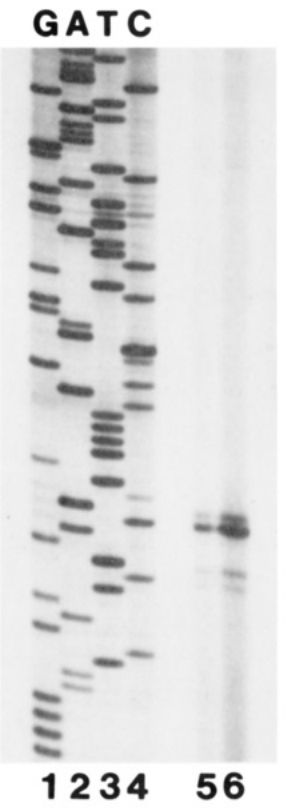

FIGURE 2: Mapping of the 5′ end of the LPL mRNA. Radiolabeled primer (see Figure 3) was annealed to 40 μg of poly(A)+ human adipose tissue RNA and extended with AMV reverse transcriptase as described under Materials and Methods. The primer extension products are seen in lanes 5 and 6 (one-fourth and half of the reaction product, respectively). The sequence ladder (dideoxy chain termination reaction) generated with the same oligonucleotide primer on a cloned DNA template (clone in Figure 1) shown in lanes 1–4 was used as a size marker. The first G from the bottom is complementary to the C in position +17 and corresponds to a fragment of 43 bases.

ATTTGCAT-3 (at −46), found in immunoglobulin gene promoters (Parslow et al., 1984) and in the heavy-chain enhancer that specifically binds the ubiquitous nuclear factor A (NF-A) (Singh et al., 1986; Sen & Baltimore, 1986); a Ca^{2+}-responsive motif 5′-TGAGGTTT-3′ (at −54) which is similar (7/8 bases) to that of the c-fos gene (Sheng et al., 1988); the 5′-GAGAGGA-3′ motif (at −355) found in the 5′ upstream region of the mouse adipsin, adipocyte P2 protein, and glycerophosphate dehydrogenase genes, which are transcriptionally induced as mouse 3T3-F442A cells differentiate into adipocytes (Distel et al., 1987).

Discussion

LPL is a member of the family of lipases that includes hepatic lipase and pancreatic lipase as evidenced by considerable homology in both structure and function (Wion et al., 1987). It is therefore likely that an ancestral lipase gene had given rise to the present family of lipase genes (Ben-Avram et al., 1986; Komaromy et al., 1987). Delineation of the structure of lipase genes would give valuable information as to their evolution. The human lipoprotein lipase gene is approximately 30 kb in length, contains 10 exons, and has an intron:exon length ratio of 9, which is typical for a mammalian gene. The length of the first nine exons of the gene (ranging from 105 to 276 bp) falls within the range generally observed in mammalian genes (Breathnach & Chambon, 1981; Gilbert, 1985). The tenth exon, which includes only the 3′ untranslated sequences, is unusually long (1.95 kb).

LPL was previously shown to have at least six functional sites or domains: a 27 amino acid signal peptide for secretion, sites for interaction with the lipoprotein substrate (interfacial binding site), for interaction with the cofactor apolipoprotein C-II, for binding to heparan sulfate, and for interaction with another subunit to form the active homodimer, and a catalytic side (Olivecrona & Bengtsson-Olivecrona, 1987; Wion et al., 1987).

The first exon contains the 5′ untranslated region followed by a sequence that codes for an apparent signal peptide which is cleaved from the protein upon secretion (Wion et al., 1987). Exon 4 codes for the domain that contains the serine (residue 132 of the mature protein) and flanking amino acid residues

−480	AAGCACAAGC	TGGGACGCAA	TGTGTGTCCC	TCTATCCCTA	CATTGACTTT	GCGGGGGTGG
−420	GGATGGGGTG	CGGGGTGAGT	GAGGGAGGAC	TGCAAGTGAC	AAACAGGATT	CGTCAAAAGA
−360	GAGGTGTATT	AAAGTGCCGA	TCAAATGTAA	TTTAACAGCT	AAACTTTCCC	TCCTTGGAAA
−300	ACAGGTGATT	GTTGAGTATT	TAACGTGAAT	CGATGTAAAC	CTGTGTTTGG	TGCTTAGACA
−240	GGGGGCCCCC	GGGTAGAGTG	GAACCCCTTA	AGCTAAGCGA	ACAGGAGCCT	AACAAAGCAA
−180	ATTTTTCCGT	CTGCCCTTTC	CCCCTCTTCT	CGTTGGCAGG	GTTGATCCTC	ATTACTGTTT
−120	GCTCAAACGT	TTAGAAGTGA	ATTTAGGTCC	CTCCCCCCAA	CTTATGATTT	TATAGCCAAT
− 60	AGGTGATGAG	GTTTATTTGC	ATATTTCCAG	TCACATAAGC	AGCCTTGGCG	TGAAAACAGT
+ 1	GTCAGACTCG	ATTCCCCCTC	TTCCTCCTCC	TCAAGGGAAA	GCTGCCCACT	TCTAGCTGCC
+ 61	CTGCCATCCC	CTTTAAAGGG	CGACTTGCTC	AGCGCCAAAC	CGCGGCTCCA	GCCCTCTCCA
+121	GCCTCCGGCT	CAGCCGGCTC	ATCAGTCGGT	CCGCGCCTTG	CAGCTCCTCC	AGAGGGACGC
+181	GCCCCGAGAT	GGAGAGCAAA	GCCCTGCTCG	TGCTGACTCT	GGCCGTGTGG	CTCCAGAGTC
+241	TGACCGCCTC	CCGCGGAGGG	GTGGCCGCCG	CCGACC		

FIGURE 3: Sequence of the first exon and 5′ region of the human LPL gene. The translational start site is overlined. The transcriptional start sites are indicated with asterisks (the preferred site with a larger asterisk is designated as +1). The TATAA (CATAA) and CCAAT boxes are indicated with lines above and below. Potential enhancer motifs for Ca^{2+} responsiveness, nuclear factor A binding (NF-A), and an octamer (ad) that seems to be involved in adipocyte specific expression of the LPL gene are underlined.

Table II: Exon–Intron Junctions in the Human LPL Gene[a]

Exon Number	Sequence of Intron–Exon Junctions: 5' Boundary	3' Boundary
1	taagcagccttggcgtgaaaacagtGTCA	GACCgtaagttttgcgcgcaaactcccct
2	ctcatatccaatttttcctttccagAAAG	GACGgtaagggaggctctttggggaagag
3	aagcttgtgtcatcatcttcagGTAA	GGAGgtaagactgggagaaggagacttat
4	acttgagcctcattctttctccaagGAGG	TACTgtaagaaagcaatttcgttggtctt
5	tgttcctgctttttttccctttaagGCCT	GGAGgtaaatattatttagaagcgaatta
6	ggaccagctagtgaagtgctcccagATGT	AAAGgtaggctggagactgttgtaaataa
7	catgttcgaatttcctccccaacagTCTT	CTCTgtgagtagctctgggggcggtcatc
8	ccaaatttattgctttttgtttagGCCT	AAAAgtaattaaatgtatttttcttcctt
9	tattcacatccatttttcttccacagGGTG	GCTGgtgagcattctgggctaaagctgac
10	ccctttttcctgagctttttctcagAAAC	CCACactaagtcattattttgtatcattt
Concencus:	$\overset{ttttttttttt}{ccccccccccc}\overset{t}{n}\overset{t}{c}agG$	$\overset{C}{A}AGgt\overset{a}{g}agt$

(Mount, 1982)

[a] The nucleotide sequences of exon–intron junctions were determined from genomic subclones by using as primers oligonucleotides corresponding to exon sequences. Exon sequences are shown in capital letters and intron sequences in lower-case letters. The amino acid codons interrupted by introns are given in Table I.

previously identified as the interfacial lipid-binding region (Federoff, 1983). Regions homologous to this domain have been noted in rat hepatic lipase, bovine lipoprotein lipase (Senda et al., 1987), porcine pancreatic lipase (Guidoni et al., 1981), and human lecithin cholesterol acyltransferase (McLean et al., 1986). Exon 5 of LPL codes for the sequence Gly^{154}–Gly^{231} of the mature protein within which are segments that also exhibit a high degree of homology among the various lipases (Komaromy et al., 1987; Wion et al., 1987; Enerbäck et al., 1987), suggesting that these may be involved in catalysis. Exons 6 and 9 code for stretches of sequences that are rich in basic amino acids (Wion et al., 1987). These domains could be responsible for binding of LPL to the acidic domain of heparan sulfate on the surface of the capillary endothelium. On the basis of heparin binding analysis of tryptic and chymotryptic fragments of LPL, Enerbäck et al. (1987) localized the heparin binding site to somewhere in between residues 256 and 397, thus excluding exon 9. These authors proposed the segment including residues 319–327 as a likely candidate for this function, as it contains five positively charged amino acids. This segment, which is located within exon 6, has the sequence KVRAKRSSK, which is similar to the postulated receptor-binding sites of apolipoproteins B and E that have the consensus sequence RXXRKRXXR/K (Innerarity et al., 1983; Yang et al., 1986). It has in fact been proposed that LPL could serve as a ligand for the apolipoprotein B/E receptor (Felts et al., 1975).

The apparent correspondence between exons and functional units of the LPL gene is consistent with the hypothesis of exon shuffling proposed as a mechanism by which new functional proteins have evolved, namely, the selection of different combinations of functional units generated by unequal crossing over in introns (Gilbert, 1978, 1985; Gō, 1983; Gilbert et al., 1986). Comparison of the positions of introns in members of the lipase family of genes in various species would help portray its evolutionary history.

The 5′ termini of the LPL mRNA from human adipose tissue were determined by primer extension analysis. There appear to be four transcription initiation sites, one of which (188 bp 5′ upstream from the ATG initiation codon) is favored. A TATA-like (CATAA) and CCAAT promoter elements, together with some potential enhancer motifs, were identified in the 5′ upstream region. One of these potential enhancer motifs (GAGAGGA at −355) may be required for adipose tissue-specific expression (Distel et al., 1987). Another (TGAGGTTT at −54) may allow transcriptional activation in response to intracellular Ca^{2+} mobilization (Sheng et al., 1988). The LPL gene is known to be strongly expressed in adipose tissue (Wion et al., 1987) and transcriptionally induced by treatment of the monocytic leukemia cell line THP-1 with the Ca^{2+} ionophore A23187 (Auwerx et al., 1989). Functional analysis of the regulatory elements of the LPL gene in a variety of cell types will be necessary to define these and other promoter and enhancer elements.

Delineation of the structure and restriction map of the LPL gene should allow localization of two major LPL gene rearrangements recently observed in a family with LPL deficiency (Langlois et al., 1989). In addition, sequences flanking the coding exons should allow rapid amplification and determination of the sequence of defective alleles generated by point mutations.

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Competitive Inhibition of Phospholipase A_2 in Vesicles†

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Received February 9, 1989; Revised Manuscript Received March 14, 1989

ABSTRACT: Kinetic studies with phospholipase A_2 are complicated by the fact that binding of the enzyme to the interface precedes catalytic turnover. This difficulty can be overcome by monitoring interfacial catalysis in the scooting mode where the enzyme does not leave the interface. The kinetics of inhibition by transition-state analogues shows that specific competitive inhibition is the result of competition between inhibitor and substrate for the binding to the active site of the enzyme in the interface. Several lipophilic compounds, including alkanols, substituted butyrophenones, aristolochic acid, and mepacrine apparently reduce the rate of lipolysis by promoting the desorption of phospholipase A_2 from the interface.

The products of hydrolysis of phospholipids in biomembranes by phospholipase A_2 (PLA) are biosynthetic precursors of several regulatory molecules in a wide range of tissues under a variety of physiological and pathological conditions. For example, arachidonic acid is the precursor for eicosanoids (Irvine, 1982) and lysophosphatidylcholine for platelet activating factor (Snyder, 1985). It is believed that inhibitors of PLA could exhibit a range of desirable pharmacological effects (Dennis, 1987). Interfacial catalysis by PLA is quantitatively described in terms of Figure 1 (Verger et al., 1973; Jain et al., 1986a; Jain & Berg, 1989). The enzyme in the water layer

†This research was supported by Grants GM-29703 (M.K.J.) and HL-36235 (M.H.G.) from the National Institutes of Health and by a grant from Sterling-Eastman to M.K.J. Mass spectra were obtained at the UCSF resource (A. L. Burlingame, Director, NIH RR01614).
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0006-2960/89/0428-4135$01.50/0