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LIPOPROTEIN LIPASE

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INTRODUCTION

Lipoprotein lipase (LPL, EC 3.1.1.34) is the major enzyme responsible for the hydrolysis of triglycerides in chylomicrons and very low density lipoproteins. Experimentally, inhibition of the enzyme with antisera leads to the accumulation of triglycerides in the plasma (40, 56). In type 1 hypertriglyceredemia, a rare heritable disease, patients have no LPL activity, plasma triglycerides are elevated, and the low density lipoproteins concentration is

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negligible. LPL is synthesized in most tissues including adipose, heart, lung, mammary gland, skeletal muscles, and kidney. In the adult liver, in most species, LPL mRNA is absent or in very low concentrations. In each tissue where it is present the enzyme is synthesized in subendothelial cells, secreted to the cell surface, and transported by an unknown mechanism to the capillary endothelial surface where it binds to heparan sulfate proteoglycans (12, 62). In the body LPL plays a directive function: The flux of fatty acids to a given tissue reflects the activity of the enzyme on the capillary bed of the tissue (54). The activity of the enzyme on the endothelium, which is often referred to as the functional enzyme, varies with the energy needs of individual tissues. In the fed state the activity in the adipose tissue is high, whereas it is low in the fasted state. In heart tissue the opposite occurs: Activity is low in the fed state and high in the fasted state. Recently a comprehensive review on the biochemistry and function of LPL was published (28). During the past three years cDNA's for LPL of several species have been isolated, and high titer antibodies for various LPLs have been characterized. With these reagents new mechanistic information has been obtained on the structure, synthesis, intracellular transport, and regulation of the enzyme in cell culture. These topics are the major subjects discussed in this review.

PURIFICATION

LPL has been purified to homogeneity from several tissues. Bovine milk is an abundant source of enzyme. From 3 liters of skim milk, 1.5 to 2 mg of enzyme is purified with a yield of 10 to 13% (36). The method utilizes affinity chromatography on heparin-Sepharose CL-6B, adsorption chromatography on Cγ-aluminum hydroxide gel, and a final intervent dilution chromatography step on a heparin-Sepharose CL-6B column. The purity of these preparations is in excess of 95%. The minor contaminants can be shown by Western blotting to be mainly proteolytic fragments. Socorro & Jackson (64) have suggested adding 1 mM phenylmethane sulfonyl fluoride to the starting skim milk to inhibit enzyme degradation. Recently, Zechner (78) devised a rapid isolation procedure of human milk LPL that utilized two sequential chromatography steps on heparin-Sepharose and phenyl-Sepharose. LPL has been purified from adipose tissue of rat (30, 51), pig (4, 43), and chicken (13) utilizing similar purification steps. Purification of LPL from postheparin plasma has been difficult to achieve owing to copurification of anti-thrombin III and LPL on heparin-Sepharose columns. This problem was resolved by the use of heparin-Agarose with low affinity for antithrombin (36) and by the use of a hydrophic chromatography step on phenyl-Sepharose (11). In the latter procedure, LPL after a heparin-Sepharose 4B step is loaded onto a phenyl-

Sepharose CL-4B column in 1 M $(NH_4)_2SO_4$/1.2 M NaCl/10 mM phosphate buffer pH 7.0; the column is then washed with a decreasing gradient of NaCl between 2 and 0.2 M in 30% glycerol, 5 mM phosphate pH 7.0. The enzyme is then eluted with 4 mM SDS, 50 mM octyl-β-D-glucopyranoside in 30% glycerol. In the previous protocol, antithrombin binds to the phenyl-Sepharose CL-4B but is eluted during the washing procedure.

Monoclonal antibodies to bovine milk LPL (64, 74) and the chicken LPL (29) have been isolated. The monoclonal antibody CAL1-11 to avian LPL was used to construct an immuno-affinity column that was employed in a one-step purification of the enzyme. Quantitative binding of an antigen to an immuno-affinity column is easily achieved. Often, however, it is difficult to desorb enzymes with high yields and in a catalytically active state. In the case of LPL, elution of active enzyme was achieved by including acetone in the desorbing buffer. When avian LPL was exposed to buffers containing up to 8% acetone for 4 h at 4°C, 89% of the enzymatic activity was recovered. The enzyme was purified with a yield of 25%, was homogenous by SDS polyacrylamide gel electrophoresis, and contained no traces of proteolytic fragments. The procedure is rapid and could be well suited, in structure-function studies, to the purification of recombinant LPL from culture media of cells transfected with plasmid carrying mutated LPL coding sequences. In these studies, small amounts of highly purified enzyme are needed for functional assays.

PROPERTIES

The classical characteristics of LPL and its distinguishing features from other lipases are its requirement for apolipoprotein CII for maximal activity, and its inhibition by 1 M NaCl. The enzyme has maximal activity between pH 8.6 and 9.0. For excellent reviews on the activation of LPL by apolipoprotein CII and on the enzyme mechanism the reader is referred to two recent reviews by Smith & Pownall (63) and Quinn et al (52).

Molecular Weight

The bovine milk LPL has been characterized extensively. Iverius & Östlund-Lindqvist (35) obtained by sedimentation equilibrium a value of 48,300 for the monomer molecular weight, under reducing conditions in 6.6 M guanidium HCl. In the same buffer conditions by analytical gel chromatography they reported an M_r of 50,800. More recently, Olivecrona et al (45) obtained by sedimentation equilibrium a molecular weight of 41,700. In these studies the partial specific volume was determined experimentally, rather than by

calculation. Olivecrona et al (45) determined partial specific volume by obtaining concentration profiles from analytical ultracentrifugation in 6 M guanidinium chloride in H_2O and ^2H_2O. In the absence of denaturing agents the native, catalytically active LPL is present as a dimer. Sedimentation equilibrium in the presence of 0.5 M $(NH_4)_2SO_4$, 0.2 M NaCl, 1 mM sodium azide, 1 mM EDTA, and 10 mM Tris-HCl pH 7.4 demonstrated that at both low (0.01 mg/ml) and high (0.25 mg/ml) initial concentrations of enzyme, a dimer was the predominant form (50). The same conclusion was reached utilizing radiation inactivation (46). The molecular size of the functional active bovine LPL was 72 kDa, which corresponds closely to that of a dimer. The same M_r was observed for a variety of conditions in which the enzyme is known to be active, namely, in the presence of high salt buffers or of lipid substrate, as a heparin-enzyme complex, or in association with a mixed micelle of 1% Triton X-100 and 0.1% sodium dodecyl sulfate. Utilizing the same technique of radiation inactivation, Garfinkel et al (27) concluded that in rat adipose and heart tissues the active LPL was present as a dimer.

Carbohydrate Composition

Bovine milk LPL contains 8.3% carbohydrate (35). Per molecule of enzyme monomer these authors reported 10 residues of mannose, 2 of galactose, 1 of glucose, 5 of *N*-acetylglucosamine, and 2 of sialic acid. Rat LPL purified from postheparin plasma contains approximately 7% carbohydrate by weight: 1.8% sialic acid, 3.2% neutral sugars, and 1.9% amino sugars (23).

Examination of the primary sequences of the bovine (61), mouse (38), human (76), guinea pig (21), and chicken (15) LPL, deduced from full-length cDNA's, reveals the presence of three sequences that conform to the consensus sequence asn-X-(thr/ser). One of these at asn259 of the human and guinea pig sequence is of the type asn-pro-ser. This sequence is rarely glycosylated in eukaryotic proteins (66). Analysis of eight tripeptide sequences (asn-pro-ser/thr) from eukaryotic glycoproteins and proteins revealed that this site was glycosylated in only one case. In the guinea pig LPL, Semb & Olivecrona (58) have shown that the mature enzyme contains one high mannose and two complex oligosaccharide chains. The number of oligosaccharide chains was established by identification of molecular species following a time course of digestion of LPL with endo-β-*N*-acetylglucosaminidase H (endo H). In these studies LPL was metabolically pulse-labeled in mature isolated adipocytes for 5 min. The number and nature (high mannose or complex) of the oligonucleotide chains was determined by identification of molecular species generated after complete digestion of immunoadsorbed LPL in adipocytes and medium, following a pulse chase protocol. The precise location of the three oligosaccharide chains has not been identified directly by isolation of glycopeptides. Semb & Olivecrona (58) have suggested that

asn43 (asn-his-ser) and asn359 (asn-asn-thr) are conjugated to oligosaccharides and that asn257 (asn-pro-ser) is not utilized. They propose instead that asn281, where the sequence is asn-arg-cys, is conjugated. The rationale put forth is that the -SH group in cysteine can form a hydrogen bond with the amide group in asparagine analogous to the -OH groups in ser and thr. Yang et al (77) determined the complete primary structure of bovine milk LPL by alignment of proteolytic peptides. The results of gas phase sequencing analyses confirmed N glycosylation at only two residues, asn44 and asn361, which correspond to asn43 and asn359 of the guinea pig LPL. Utilizing the same methodology as that employed for the guinea pig LPL, Hoogewerf & Bensadoun (33) demonstrated that chicken LPL contains 2 complex and 1 high mannose N-linked oligosaccharide chains. The tryptic peptide containing asn45 was isolated and shown to contain a complex oligosaccharide chain. Similar studies with 3T3-F442A mouse adipocyte cell line indicate that the mouse LPL has two N-linked complex oligosaccharide chains (70).

Lipoprotein Lipase Is a Sulfated Glycoprotein

Several reports suggest that posttranscriptional and posttranslational events may be significant in the regulation of LPL (see below, under regulation). These findings have stimulated a search for posttranslational modifications such as phosphorylation, sulfation, and proteolytic cleavage that may be modulated by hormones and other factors. The only posttranslational modification reported at this time is sulfation. Chicken LPL is a sulfated glycoprotein (33). The sulfate moiety is associated with a complex oligosaccharide chain. Tryptic digestion of $^{35}SO_4$-labeled LPL and isolation of peptides by HPLC identified a single $^{35}SO_4$-labeled tryptic peptide. The amino acid sequence of the peptide established that the $^{35}SO_4$-oligosaccharide is conjugated at asn45. Sequential exoglycosidase digestion combined with the gas liquid chromatography analysis of the released sugars established that the sulfate moiety is located in a core *N*-acetylglucosamine. Utilizing the specificity of endo-β-*N*-acetylglucosaminidase D which cleaves N-linked oligosaccharides between the two *N*-acetylglucosamine residues of the chitobiose core, it was demonstrated that the sulfate is localized in the proximal *N*-acetylglucosamine. The time course of hydrolysis of oligosaccharide-bound sulfate to barium-precipitable sulfate by 0.25 N HCl at 100°C displays a first order process with a $t_{1/2}$ of 135 min, indicative of a single class of primary sulfate groups in an ester linkage to the oxygen of C6. The biological significance of sulfation in N-linked oligosaccharides is still unknown. The role of mannose-6-phosphate in targeting lysosomal enzymes to the lysosomes is well established (73). Sulfation of the innermost core *N*-acetylglucosamine residue of LPL may play a role in targeting the enzyme to the plasma membrane.

MOLECULAR BIOLOGY

During the last three years LPL cDNA clones have been isolated for several mammalian species: man (76), mouse (38), bovine (61); guinea pig (21), and chicken (15). When RNA from several human tissues was probed with cloned, labeled LPL cDNA, two bands of roughly equal intensity and corresponding to mRNA species of 3350 and 3750 nt were identified (76). The mRNA species includes, about 175 nt upstream of the translation initiation codon, a region 1425 nt long coding for a 475 amino acid protein, a termination codon TGA, and an untranslated 3' region of 1557 nt or 1952 nt followed by a poly A tail. Based on the sequence of the amino-terminal region (3), the signal peptide is 27 amino acids. The human cDNA sequence predicts a mature molecular weight of 50,394. If one assumes an 8% carbohydrate content, the predicted molecular weight is approximately 54,800. Human, bovine, guinea pig, and mouse LPL exhibit an almost complete sequence conservation. The chicken LPL sequence exhibits overall identities of 73 to 77% with the four known mammalian sequences (Figure 1).

LPL belongs to a gene family that includes hepatic and pancreatic lipases (76). Human LPL is 46% homologous with rat hepatic lipase and 28% homologous with porcine pancreatic lipase. The homology between these three enzymes is greatest in the central portions of the molecules and was referred to as the "central homology region" by Komaromy & Schotz (39). All three lipases have 10 cysteine residues that are similarly located. In the case of bovine LPL it was shown experimentally that all cysteine residues are disulfide bonded (77). Lingual lipase (38) and hormone-sensitive lipase (32) show little or no homology with LPL. Portions of the primary sequence of LPL that are highly conserved among the five species are probably significant functional and structural domains. Davis et al (18) reported that mutagenesis of ser147 in human hepatic lipase yields a protein with little or no hydrolytic activity with either triolein or tributyrin. Mutagenesis at ser133 or ser228 had no effect on catalysis. The interfacial lipid-binding region of porcine pancreatic lipase had been shown by chemical modification with p. nitrophenyl phosphate to reside around ser152 (31). Recently, Winkler et al (75) determined the three-dimensional structure of human pancreatic lipase. Structural results provide evidence that ser152 is the residue essential for catalysis. This putative hydrolytic site is chemically similar to the catalytic asp-his-ser triad characteristic of serine proteases. One can therefore infer that ser132 of human LPL, which corresponds to human pancreatic lipase ser152, is probably essential for catalysis. Similarly by sequence homology, asp156 and his241 are the putative residues in the catalytic triad. The four mammalian sequences have three potential N-glycosylation sites (asn-X-ser/thr). In the

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cLPL    SDPEAEMNFEGIESKFSLRTPAEPDEDVCYLVPGQMDSLAQCNFNHTSKTFVVIHGWTVT   60
bLPL    DRITGGKDFRDIESKFALRTPEDTAEDTCHLIPGVTESVANCHFNHSSKTFVVIHGWTVT   60
mLPL    -AADAGRDFSDIESKFALRTPEDTAEDTCHLIPGLADSVSNCHFNHSSKTFVVIHGWTVT   59
hLPL    --ADQRRDFIDIESKFALRTPEDTAEDTCHLIPGVAESVATCHFNHSSKTFMVIHGWTVT   58
gpLPL   --ANCQKDYTDIESKFARRTPENTVEDTCHLIPGVTESVANCHFNHSSKTFMVIHGWTVT   58

cLPL    GMYESWVPKLVDALYKREPDSNVIVVDWLVRAQQHYPVSAAYTKLVGKDVAMFIDWMEEK   120
bLPL    GMYESWVPKLVAALYKREPDSNVIVVDWLSRAQQHYPVSAGYTKLVGQDVAKFMNWMADE   120
mLPL    GMYESWVPKLVAALYKREPDSNVIVVDWLYRAQQHYPVSAGYTKLVGNDVARFINWMEEE   119
hLPL    GMYESWVPKLVAALYKREPDSNVIVVDWLSRAQEHYPVSAGYTKLVGQDVARFINWMEEE   118
gpLPL   GMYESWVPKLVAALYKREPDSNVIVVDWLRRAQHHYPESADYTKLVGEDVARFINWMEDE   118

cLPL    FNYPLNNVHLLGYSLGAHAAGIAGSLTKKKVNRITGLDPAGPTFEYADAPIRLSPDDADF   180
bLPL    FNYPLGNVHLLGYSLGAHAAGIAGSLTNKKVNRITGLDPAGPNFEYAEAPSRLSPDDADF   180
mLPL    FNYPLDNVHLLGYSLGAHAAGVAGSLTNKKVNRITGLDPAGPNFEYAEAPSRLSPDDADF   179
hLPL    FNYPLDNVHLLGYSLGAHAAGIAGSLTNKKVNRITGLDPAGPNFEYAEAPSRLSPDDADF   178
gpLPL   FNYSVDNVHLLGYSLGAHAAGVAGSRTNTKVSRITGLDPAGPNFEYAEATSRLSPDDAQF   178

cLPL    VDVLHTYTRGSPDRSIGIQKPVGHIDIYPNGGGFQPGCNLGEALRLIAEKGFSDVDQLVK   240
bLPL    VDVLHTFTRGSPGRSIGIQKPVGHVDIYPNGGTFQPGCNIGEALRVIAERGLGDVDQLVK   240
mLPL    VDVLHTFTRGSPGRSIGIQKPVGHVDIYPNGGTFQPGCNIGEAIRVIAERGLGDVDQLVK   239
hLPL    VDVLHTFTRGSPGRSIGIQKPVGHVDIYPNGGTFQPGCNIGEAIRVIAERGLGDVDQLVK   238
gpLPL   VDVLHTFTRGSPGRSIGIQKPVGHVDIYPNGGSFQPGCNIQDALRVISQKGFGDMDQLVK   238

cLPL    CSHERSIHLFIDSLLYEEKPSMAYRCNTKEAFEKGLCLSCRKNRCNNLGYKVNRVRTKRN   300
bLPL    CSHERSVHLFIDSLLNEENPSKAYRCNSKEAFEKGLCLSCRKNRCNNMGYEINKVRAKRS   300
mLPL    CSHERSIHLFIDSLLNEENPSKAYRCNSKEAFEKGLCLSCRKNRCNNLGYEINKVRAKRS   299
hLPL    CSHERSIHLFIDSLLNEENPSKAYRCSSKEAFEKGLCLSCRKNRCNNLGYEINKVRAKRS   298
gpLPL   CSHERSIHLFIDSLLNEENPSKAYRCNSKEAFEKGLCLSCRKNRCNNVGYEINKVRAKRS   298

cLPL    TKMYLKTRAQMPYKVFHYQVKIHFFGKTNVTKVDQPFLISLYGTLDESENIPFTLPEVSS   360
bLPL    SKMYLKTRSQMPYKVFHYQVKIHFSGTESNTYTNQAFEISLYGTVAESENIPFTLPEVST   360
mLPL    SKMYLKTRSQMPYKVFHYQVKIHFSGTENGKQHNQAFEISLYGTVAESENIPFTLPEVST   359
hLPL    SKMYLKTRSQMPYKVFHYQVKIHFSGTESETHTNQAFEISLYGTVAESENIPFTLPEVST   358
gpLPL   SKMYLKTRSQMPYKVFHYQVKIYFSGTETTTYTNQAFEISLYGTVAESENIPFTLPEVSA   358

cLPL    NKTFSFLIYTEVDIGDLLMLKLQWEKDTFFSWSDWWTPFAFTIQRVRVKSGETQKKVVFC   420
bLPL    NKTYSFLLYTEVDIGELLMLKLKWISDSYFSWSNWWSSPGFDIGKIRVKAGETQKKVIFC   420
mLPL    NKTYSFLIYTEVDIGELLMMKLKWMSDSYFSWPDWWSSPSFVIERIRVKAGETQKKVIFC   419
hLPL    NKTYSFLIYTEVDIGELLMLKLKWKSDSYFSWSDWWSSPGFAIQKIRVKAGETQKKVIFC   418
gpLPL   NNTYSFLIYTEVDIGELLMLKLKWITESYFSWSSWWGRPTFTIEKIRVKAGETQKKIVFC   418

cLPL    SRDGSSRLGKGEEAAIFVKCLEQPVSRKRGGAKKASKENSAHESA   465
bLPL    SREKMSYLQKGKSPVIFVKCHDKSLNRKSG----------------   450
mLPL    AREKVSHLQKGKDSAVFVKCHDKSL-KKSG----------------   448
hLPL    SREKVSHLQKGKAPAVFVKCHDKSLNKKSG----------------   448
gpLPL   SREKVSKLQKGKEAPVFVKCHDKSLNKKSG----------------   448
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Figure 1 Aligned amino-acid sequence predicted from lipoprotein lipase cDNA clones for chicken (cLPL), bovine (bLPL), mouse (mLPL), human (hLPL), and guinea pig (gpLPL). Conserved amino acids are indicated by a dot and conserved cysteines by an asterisk overhead. Potential N-glycosylation sites are underlined and the putative catalytic region double underlined.

milk LPL it was shown by direct sequencing that only two of these sites at asn44 and asn361 are utilized (77). Asn259 is not utilized. This asn residue in mammalian LPLs is part of the tripeptide asn-pro-ser, which is rarely glycosylated in eukaryotic proteins (66). In the chicken LPL the location of the glycosylation site in the middle of the molecule at asn329 has not been conserved in contrast to the other two glycosylation sites that are in perfect registry in the five lipases on Figure 1.

The heparin-binding domain of LPL has not been established experimentally. Martin et al (42) by analogy with the heparin-binding regions of apolipoprotein E and apolipoprotein B proposed several regions in LPLs that would conform to the consensus sequences B_1-B_2-B_3-X-X-B_4 or B_1-B_2-X-B_3 in which B is a basic residue and X is a hydropathic residue. The sequence RKNR starting at residue 279 of human LPL is conserved in all five lipases. In the chicken LPL at residue 148, a sequence KKKVNR conforms to the consensus sequence B_1-B_2-B_3-X-X-B_4. Clearly the heparin-binding domain of LPL as well as the sites involved in interaction with apolipoprotein CII and in homodimer formation need to be defined experimentally.

The human LPL gene has been cloned and characterized (37). It is composed of 10 exons and spans approximately 30 kilobases. The first exon encodes the 5'-untranslated region and the signal peptide. The following eight exons encode the mature protein, and the tenth exon the very long untranslated region. Enerbäck & Bjursell (20) have characterized the guinea pig LPL gene. For a stretch of 18 amino acids coded on exon 6 of the guinea pig LPL gene, they noted an interesting homology with a similar amino acid sequence encoded by the first exon of rat fatty acid binding protein. The amino acid sequence between residues 257 and 274 of guinea pig LPL may contain a putative fatty acid binding domain first implied by observations on the binding of fatty acids to the lipase (2).

BIOSYNTHESIS, SECRETION, AND DEGRADATION OF LPL IN CULTURED CELLS

Studies on the regulation of LPL in whole animals have yielded significant information on the differential regulation of the enzyme in various organs by hormones and nutritional status (16, 28, 44). With the recent availability of well-characterized antibodies and cDNA probes, it became possible to start to dissect, in isolated cells, the various steps in the molecular regulation of synthesis, intracellular transport, and secretion of the enzyme. Most of these studies have utilized isolated cell lines that could be differentiated in culture to adipocytes or mature adipocytes isolated from adipose tissue by the collagenase procedure of Rodbell (55). Another approach has been to isolate the

somatovascular fraction from adipocytes and differentiate the cells in culture (68). Finally, cultured heart cells have also been employed productively by the Steins and their group (26).

Secretion of the Enzyme

Quantitative studies of the steady state secretion of LPL by cells in culture has been complicated by the difficulty in assaying LPL activity in crude cell extracts, and by the loss of activity at 37°C as enzyme protein is exported to the medium. Vannier et al (72) have shown with morphological and biochemical evidence that LPL is localized mainly in the Golgi, thereby establishing that the enzyme is a secretory protein. In cell extracts prepared in the absence of detergents, enzyme activity is systematically underestimated (71), which strongly implies that all the activity is present in a latent state within cisternae. In addition, in cell extracts prepared with detergents, full expression of enzyme activity is only achieved after extensive dilution of the cell extract. The problem of inactivation of the secreted enzyme in the medium has been resolved by placing cells in a perfusion chamber and stabilizing LPL in the perfusing buffer by glycerol addition and lowering of the temperature to 4°C (69). Another approach to quantitatively measure enzyme secretion has been to measure enzyme protein by radioimmunoassay or ELISA (13, 14).

A time course of secretion of LPL activity by Ob17SA16 or 3T3-F442A mouse adipose cell lines (71) measured by the continuous flow technique shows a biphasic function. The first phase, which is independent of protein synthesis, was interpreted to correspond to the secretion of a preexisting pool of intracellular molecules. The early phase lasts approximately 40 min. Enzyme released in the medium likely originates from a plasma membrane pool in equilibrium with an intracellular pool of enzyme contained in transport vesicles. The second phase during which secretion occurs at a lower rate corresponds to a constitutive secretion of enzyme. Careful catalytic activity measurements of cell extracts and medium under conditions where enzyme degradation is negligible and protein synthesis is inhibited by cyclohexamide have convincingly demonstrated (71) that no activation of enzyme activity occurs as the protein is secreted. Robinson et al (53) had suggested that LPL exists in the cell as an inactive precursor.

Intracellular Transport and Acquisition of Catalytic Activity During Processing

As a glycoprotein, LPL must be synthesized on membrane-bound ribosomes and translocated into the lumen of the endoplasmic reticulum. Two issues that have attracted considerable interest are (*a*) is glycosylation of the enzyme necessary for secretion and catalytic activity, and (*b*) at what stage during the

processing of the enzyme oligosaccharide chains is catalytic activity acquired? Several reports indicate that glycosylation of the enzyme is necessary for its activity and synthesis. Chajek-Shaul et al (8) have shown in rat preadipocytes and heart cells that inhibition of N-glycosylation with tunicamycin inhibited cellular LPL activity. With both cell types, LPL activity could not be detected in the medium. Similar results have been reported with 3T3-L1 (47) and murine Ob17 adipocytes (1). In the latter experiments with Ob17 cells, it was also demonstrated that immunoreactive enzyme is secreted in the medium. Glycosylation has also been blocked in cultured rat adipocytes by maintaining cells in glucose-depleted medium (48). In recent experiments, Semenkovich et al (59) utilizing site-specific mutagenesis have narrowed the requirement for glycosylation in human LPL to the N-linked oligosaccharide at asn43. Intracellular and secreted LPL activity was absent when Ala was substituted for asn at position 43. However, mutations at asn257 and 359 did not affect activity.

Recently, Semb & Olivecrona (58) in studies with guinea pig LPL addressed the question, when does the enzyme acquire catalytic activity during its processing? In this species the mature enzyme contains one high mannose and two complex oligosaccharide chains. When adipocytes were pulsed for 10 min and a cell extract applied to a heparin Sepharose column, LPL activity eluted at 1 M NaCl. In addition, if the peak fractions with enzyme activity were immunoprecipitated and characterized with respect to endo H-sensitivity, the fully endo H-sensitive species with three high mannose chains co-eluted with the mature form with a single high mannose chain. Because only the catalytically active LPL species, in a dimeric form, is known to bind to heparin-Sepharose, the implication was that the fully endo H–sensitive form of the enzyme existed as a dimer and was active. The latter point was established directly by separating the endo H–sensitive LPL species on lectin affinity columns and showing that they displayed catalytic activity in proportion to their content of immunoprecipitable enzyme protein. The same authors showed that inhibition of mannosidases 1A and 1B with deoxymannojirimycin or of glucosidases I and II with methyldeoxynojirimycin led to the secretion of endo H-sensitive LPL, which was catalytically active. Therefore, trimming and processing of oligosaccharides is not necessary for acquisition of catalytic activity or for secretion of LPL in guinea pig adipocytes. Differences exist among species with respect to requirements for LPL homodimer formation. In adipose 3T3-F442A cells Vannier & Ailhaud (70) have shown by sucrose gradient centrifugation that the species (M_r = 55,000) that is found in the endoplasmic reticulum and is fully endo H-sensitive is present as an inactive monomer, whereas the mature species (M_r = 58,000) bearing two N-linked complex oligosaccharides is present as an active homodimer. Whether a true difference in maturation exists between mouse LPL and

guinea pig LPL or whether it is a peculiarity of the 3T3-F442A cell line needs to be determined.

Translocation of LPL from intracellular pools to the cell surface, i.e. the heparin releasable pool, appears to be stimulated by β-adrenergic stimulation. Acute treatment (3 min) of cultured rat heart cells (25) by Bt_2 cAMP or isoproterenol results in a threefold increase in heparin-releasable LPL with a concomitant decrease in cell-associated enzyme.

Degradation of Lipoprotein Lipase in Adipocytes

Several groups independently have shown with isolated mature fat cells or fat cells differentiated in vitro (17, 47, 57, 70) that 70 to 80% of the newly synthesized LPL is degraded before appearing in the medium. This was a very significant observation because it raised the possibility that in vivo rapid modulation of enzyme output could occur without changes in enzyme rate of synthesis. A maximal potential fourfold increase in enzyme secretion could be explained by this mechanism. Extensive LPL degradation in adipocytes is not a cell culture artifact. It was also observed by Speake et al (65) in rat fat pads. Degradation is essentially inhibited when heparin is added to the medium. Quantitative measurements of LPL turnover can be calculated from pulse-chase experiments. Figure 2 illustrates the results of an experiment in which cells were pulsed for 60 min and chased for 60 min in the presence or absence of heparin (17). During the 60-min chase, 76 ± 7% of the intracellular [^{35}S]methionine-labeled LPL had been secreted or degraded (Figure 2*A*); this figure was increased to 92 ± 1% in the presence of heparin. Analyzing the same data as a semilogarithmic plot reveals that labeled LPL is lost from the cell by an apparent first order process with no evidence of concentration of LPL in storage vesicles. In the presence of heparin, very little LPL is associated with the cell surface (Figure 2*B*). After a 60-min chase, the radiolabeled LPL in the medium was 5-fold higher in heparin-treated cells (Figure 2*C*). The first order disappearance of radiolabeled LPL from the intracellular compartment is due to both secretion and degradation. Under steady state conditions, the synthesis rate can be estimated as the product of the intracellular pool size and the first order rate constant. These calculations indicate that the synthesis rate in control cells is not different from that observed in heparin-treated cells (17). Secretion rates can be measured directly by ELISA or from the radioactivity in the medium LPL pool and the specific activity of the precursor pool.

$$S = \frac{M_{60}}{\int_0^{M_{60}} y \, dt}$$

where S is the secretion rate, M_{60} the radioactive LPL accumulating in the medium in 60 min, and y the function describing the specific activity of the immediate precursor pool of the secreted enzyme. The cell surface LPL compartment is the immediate precursor pool. The average secretion rates for three experiments similar to those described in Figure 1 were 1.9 ± 0.8 and 7.2 ± 3.6 ng LPL/hr, respectively, for control and heparin-treated cells. The differences are statistically significant when analyzed as paired observations. Degradation rates calculated as the difference between synthesis and degradation were 6.2 ± 2.5 and 1.9 ± 1.8 ng LPL/hr, for the control and heparin-treated cells. The striking result in the above turnover experiment is that in control cells 77% of the synthesized enzyme was degraded, whereas in heparin-treated cells this was reduced to 21%.

On the basis of the above results the following hypothesis has been proposed (14, 17) to describe the secretion of LPL in cells and the mechanism

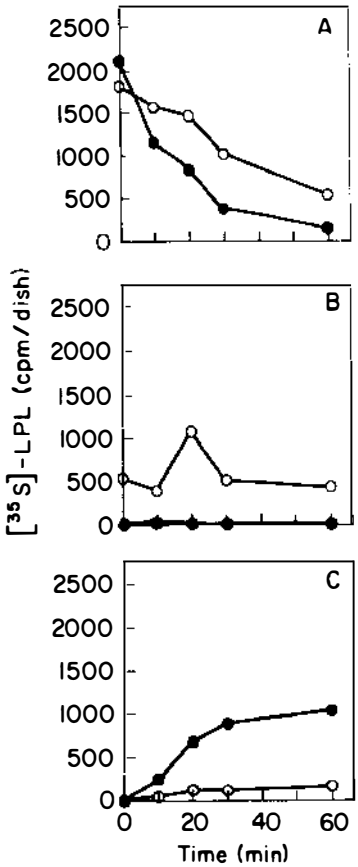

Figure 2 Secretion of labeled lipoprotein lipase during a 60-min chase in the presence of control medium (*open circle*) or medium containing 10 U/ml heparin (*solid circle*). Adipocytes were radiolabeled with L-[^{35}S]methionine (100 μCi per dish, 5 μM L-methionine) for 60 min at 37°C and then chased for the indicated time in label-free medium containing 105 μM L-methionine. Radiolabeled lipoprotein lipase was isolated by immunoadsorption followed by electrophoresis and fluorography. The data shown are the results from three similar experiments; *A*, cell extract; *B*, heparin-releasable surface-associated lipoprotein lipase; and *C*, medium ^{35}S-labeled lipoprotein lipase. Each value was derived from a single pool of five dishes of adipocytes. (Data from reference 17.)

of action of heparin on LPL degradation. This hypothesis is depicted in Figure 3. LPL is transported to the adipocyte cell surface where it binds to heparan sulfate proteoglycan (HSPG) molecules. Heparin added to the medium would compete with HSPG, extract LPL from the cell surface pool, and form LPL-HSPG soluble complexes in the medium. In control cells, in the absence of heparin a fraction of LPL bound to the cell surface is released to the medium or is internalized as an LPL-HSPG complex. A fraction of the internalized LPL is degraded in the lysosomal compartment, and the balance is recycled to the cell surface. The following lines of evidence support the hypothesis: (*a*) high affinity binding sites are on the adipocyte cell surface (14, 24); (*b*) LPL bound to adipocytes is, in part, internalized and degraded in the lysosomal compartment (14, 24) or recycled to the cell surface (14); (*c*) degradation of adipocyte cell surface heparan sulfate proteoglycans with heparinase or heparitinase decreases binding and internalization of LPL by adipocytes and secondarily results in increased LPL output in the medium (14); (*d*) finally, consistent with the proposed hypothesis, the intracellular pool size of LPL decreases under all conditions where interaction of LPL with cell surface heparan sulfate proteoglycans (HSPG) is inhibited. Under conditions where internalization is inhibited one would expect that less enzyme would be present in endosomes and in recycling vesicles. Exposure of adipocytes to heparin results in a 38% decrease in intracellular LPL, whereas the cell surface associated enzyme protein is reduced to less than 10% of control values (17). Similarly in adipocytes treated with heparinase or heparitinase, the cell surface and intracellular LPL compartments are greatly reduced (14). The model presented above suggests that no significant degradation of enzyme molecules occurs during the intracellular transport of enzyme to the cell surface and that internalization in association with heparan sulfate proteoglycans is needed for degradation to occur. A similar interpretation of the effect of heparin on the degradation of LPL in isolated guinea pig fat cells was offered by Semb & Olivecrona (57). Conrad and his group (6, 34) have shown in studies with a rat hepatocyte cell line that hepatocytes secrete HSPG into the cell surface matrix and the medium and internalize them from both compartments. The same group also discovered that treatment of cultured hepatocytes with phosphatidylinositol-specific phospholipase C stimulates the release of HSPG into the medium (34). The authors postulated that the released HSPG molecules would bind to a putative inositol phosphate receptor. In support of this hypothesis myo-inositol hexaphosphate was shown to enhance the release of HSPG into the medium. In chick adipocytes phosphatidylinositol-specific phospholipase C stimulated the release of HSPG and, consistent with the existence of an inositol phosphate receptor, inositol phosphate enhanced LPL secretion in cultured adipocytes (L. Cisar and A. Bensadoun, unpublished data).

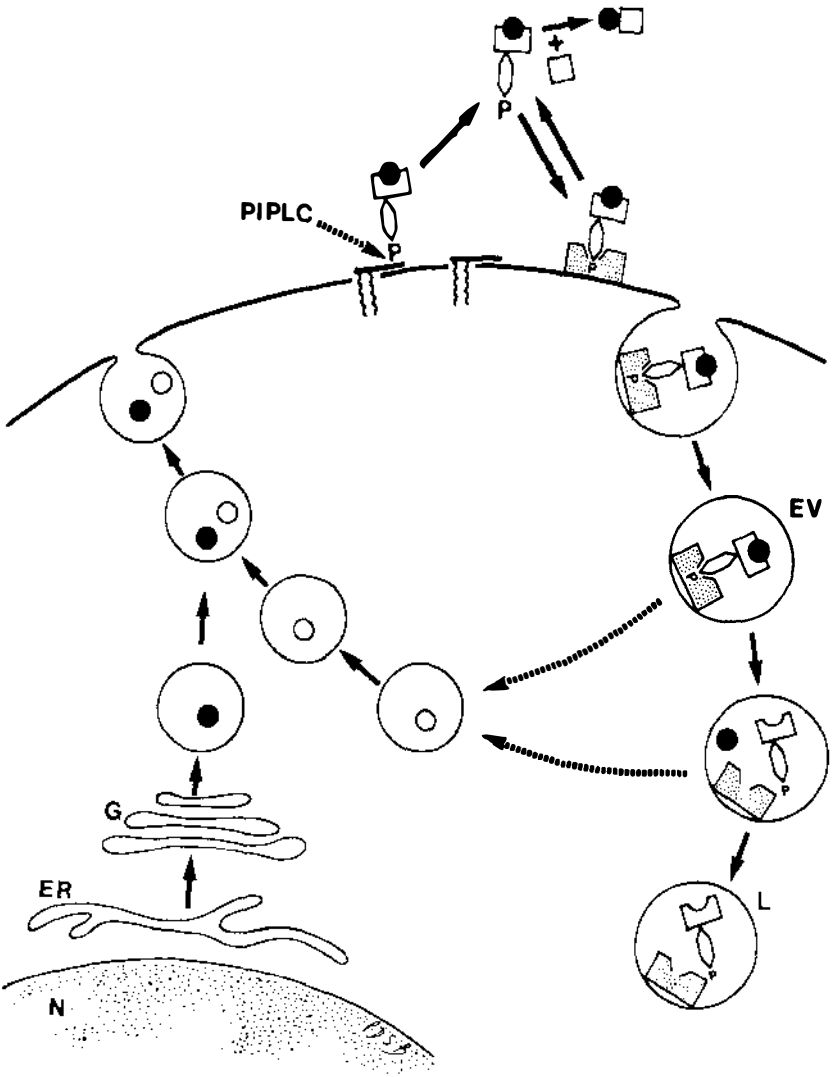

Figure 3 Proposed pathway of lipoprotein lipase. *Closed circle* denotes newly synthesized LPL; *open circle*, recycled LPL; *square*, molecules with high affinity for LPL, such as heparin or lipoproteins. The newly synthesized LPL is shown bound to a HSPG anchored via a phosphatidyl-inositol moiety. After release from the cell surface the HSPG would be recognized by an inositol phosphate receptor, shown as the stippled form on the cell surface; EV, endocytic vesicle and L, lysosome.

In their studies of LPL turnover in 3T3-F442 A cells, Vannier & Ailhaud (70) present a different model of intracellular transport of the enzyme. At the *trans*-side of the Golgi apparatus LPL molecules present as active homodimers are directed to either a constitutive pathway or a regulated pathway. These two pathways contribute enzyme molecules to two distinct compartments C (constitutive) and R (regulated). Efflux from the R pool leads mainly to lysosomal degradation, whereas efflux from C accounts for the constitutive secretion. Heparin or other secretagogues stimulate exocytosis from R while the flux of enzyme to the lysosomal compartment is now negligible. Both models should be further tested experimentally.

Is LPL Anchored to Cells via a Phosphatidylinositol Anchor?

Chan et al (10) proposed on the basis of studies conducted with 3T3-L1 cells differentiated to adipocytes that LPL in this cell type was anchored to the plasma membrane via a phosphatidylinositol moiety. The conclusion was based on the following evidence: In cultured differentiated 3T3-L1 cells LPL is released in the medium upon treatment of cells with phosphatidylinositol-specific phospholipase C purified from *Bacillus thuringiensis;* it was also shown by immunoprecipitation that [^{32}P]orthophosphate and [^{3}H]glucosamine are incorporated into immunoprecipitated LPL. Both components are known constituents of glycosylphosphatidyl anchors (41). Finally, the shift in solubility of cell surface LPL from a Triton X-114 soluble to a Triton X-114 insoluble species after treatment with phosphatidylinositol phosphate-specific phospholipase C was also taken as evidence that LPL is a phosphatidyl inositol–anchored protein. This report has not been confirmed. Chajek-Shaul et al (9) studied the release of LPL in cultured rat heart cells with exogenous phosphatidylinositol-specific phospholipase C. Like Chan et al (10) they observed an enhanced LPL release with phospholipase treatment. They also reported that if rat cells were first pretreated with heparin, no LPL was released by the bacterial phospholipase C. In addition they observed that 35S-labeled proteoglycans were released by the phospholipase. Chajek-Shaul et al (9) concluded that the release of LPL by phosphatidylinositol-specific phospholipase C is due to the release of heparan sulfate to which LPL is bound. Ting & Pagano (67) have recently reported on the presence of a calcium-sensitive phosphatidylinositol-specific phospholipase C at the external cell surface of Swiss 3T3 cells. This phospholipase C may also exist in adipocytes and be subject to hormonal regulation, thereby regulating the release of LPL-heparan sulfate proteoglycan complexes.

REGULATION OF LPL ACTIVITY IN CULTURED CELLS

This section discusses solely the regulation of LPL in cultured cells. For coverage of the in vivo regulation of the enzyme, the reader is referred to

recent reviews (7, 28). This discussion is limited to experiments that have utilized recently available immunological and cDNA probes. The central role of LPL in triglyceride transport leads one to believe that the enzyme is finely regulated and that several levels of regulation coexist in various tissues. The major hormonal modulators of the enzyme include insulin, glucocorticoid, thyroxine, glucagon, and other hormones affecting intracellular Bt_2 CAMP (28). Insulin is considered a major positive effector of LPL (54). Ong et al (49) measured the effects of insulin on the synthesis of LPL in isolated primary adipocytes. Synthesis rate was estimated by incorporation of [^{35}S] methionine into LPL during a 20- to 30-min pulse. When cells were exposed to insulin for 2 hr, insulin stimulated a maximal dose-related increase in synthesis of 300% of control. Two-thirds of the increase in synthesis rate could be accounted for by an increase in LPL mRNA content in the fat cell. The authors also measured the rate of disappearance of radiolabeled cellular LPL during a 120-min chase following a 30-min pulse and found that it was not affected by insulin. Semenkovich et al (60) employed similar techniques with mouse 3T3-L1 cells that had been differentiated into adipocytes in culture. In this instance, LPL activity measured in three compartments (intracellular, cell surface, or medium) in cells exposed to 10^{-6} M insulin was higher than in controls, but there was no corresponding increase in LPL mRNA or transcription rate. Similarly, insulin decreased the LPL protein synthetic rate, measured by immunoprecipitation, by 42–48%. Semenkovich et al (60) concluded that in the differentiated 3T3-L1 adipocytes, insulin regulation of LPL occurs entirely at posttranscriptional and posttranslational levels. The conflicting results in these two studies are probably due to the fact that the two models are greatly different. The differentiated 3T3-L1 cell line may have lost the capacity to respond to insulin.

In a recent study on the response of LPL to feeding and fasting in rats, Doolittle et al (19) concluded that LPL is controlled posttranslationally. After fasting, LPL mRNA levels and rates of synthesis increased nearly twofold. These authors measured the half-life of LPL in the endoplasmic reticulum and the Golgi/post-Golgi secretory pathway by differentiating between LPL high mannose and complex forms. In the endoplasmic reticulum LPL disappeared with a half-life of 40 min in both fed and fasted rats. However, the catabolic rate constant in the Golgi/post-Golgi compartment was 3.5-fold greater in fasting rats. In the model proposed by Doolittle et al (19), synthesis rate is constant and enzyme is either secreted, during feeding, or diverted to a degradation pathway during fasting. When the effect of feeding was studied in the guinea pig and the chicken, regulation at the level of mRNA content was observed. In guinea pig adipose tissue, Enerbäck et al (22) reported that LPL mRNA was 82% lower in animals fasted for 48 hr. Similarly, in chicken fasted for 48 hr and then refed, LPL mRNA levels in adipose increased

gradually to a maximal level at 10 hr that was 350% that of controls (15). Why the results of the last two experiments differ so greatly from those of Doolittle et al (19) is not clear. Species differences can be invoked. In addition, the fasting/feeding protocols, as well as the sampling times after feeding, differed. Heart cell LPL activity has been shown to be up regulated in culture by Bt_2 (dibutyryl) cAMP or cholera toxin (9, 26). In cultured adipocytes, up to 8 hr after addition of Bt_2 cAMP, a gradual decrease in secreted activity to 20% of control levels was observed (26). In avian-cultured adipocytes (5), utilizing a radioimmunoassay, it was shown that Bt_2 cAMP decreases both the cellular enzyme content and the enzyme catalytic efficiency. In the presence of 0.5 mM theophylline, the Bt_2 cAMP-mediated decrease in LPL activity was half-maximal at less than 25 μM Bt_2 cAMP. By 2 hr after exposure of adipocytes to 0.5 mM Bt_2 cAMP the relative synthesis rate, estimated by immunoadsorption, had decreased to 64% of control values. After 16 hr the synthesis rate was 43% of the control rate. At that time, however, cellular activity was less than 10% of the control values. Clearly, the decrease in synthesis rate only partially accounts for the observed differences in catalytic activities, and therefore other mechanisms like posttranslational events may be significant. This brief review of recent work on the regulation of LPL at the cellular level suggest that LPL is regulated at a variety of levels including transcription, translation, and posttranslational processing.

CONCLUSION

This review has largely emphasized results obtained during the last four years. After the isolation of cDNA clones and antibodies for LPLs from different species, deduced sequences became available for analysis and mechanistic studies on the molecular regulation of LPL in cell culture systems were initiated. Site-directed mutagenesis is being used successfully in the identification of the various domains of the enzyme. The challenge in future years may be to develop methods for the study of regulation of LPL in the whole organism. Commonly used measurements like postheparin plasma lipolytic activity and tissue activities may need to be replaced by more refined physiological measurements of the capacity of a tissue or whole organism to hydrolyze chylomicron and VLDL triglycerides.

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