Lipoprotein lipase and hepatic lipase: the role of asparagine-linked glycosylation in the expression of a functional enzyme

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Abstract Lipoprotein lipase (LPL) and hepatic lipase (HL) share two conserved asparagine-linked glycosylation sites, located at the amino- and carboxy-terminal domains of the protein. Human HL contains two additional sites, preceding each conserved site by 36 and 35 amino acids, respectively. The utilization of these sites for glycan-binding and the role of each glycan chain for the catalytic function of human LPL, rat HL, and human HL was investigated. To accomplish this aim, potential Asn glycosylation sites were changed to Gln by site-directed mutagenesis and the resulting constructs were expressed in a mammalian (COS) cell system. We demonstrate the following. *1)* All potential glycosylation sites in human LPL, rat HL, and human HL are utilized. *2)* Lack of glycosylation at the two nonconserved sites in human HL has no effect on enzyme expression. *3)* Glycosylation at the conserved Asn sites in the N-terminal domain of LPL and HL is required for the synthesis of a fully active and secreted lipase. While this is an absolute requirement for LPL, a portion (approximately 25%) of HL molecules lacking glycosylation at this essential site still becomes active and secreted. However, the simultaneous elimination of both glycosylation sites at the N-terminal domain of human HL results in the virtual abolishment of enzymatic activity and secretion. *4)* Glycosylation at the conserved sites in the C-terminal domain is not essential for the expression of active lipases. *5)* Eliminating all glycosylation sites in LPL and HL results in the synthesis of inactive enzymes that are retained intracellularly; however, a small portion (2%) of unglycosylated rat HL was active and secreted. We conclude that glycosylation overall plays an important role in the formation of functional LPL and HL.—**Ben-Zeev, O., G. Stahnke, G. Liu, R. C. Davis, and M. H. Doolittle.** Lipoprotein lipase and hepatic lipase: the role of asparagine-linked glycosylation in the expression of a functional enzyme. *J. Lipid Res.* 1994. **35:** 1511–1523.

Supplementary key words lipase gene family • site-directed mutagenesis • N-terminal domain • C-terminal domain

Lipoprotein lipase (LPL) is an endothelial-bound enzyme responsible for the triglyceride hydrolysis of circulating chylomicrons and very low density lipoproteins. For maximal activity, the enzyme requires the cofactor apolipoprotein (apo) C-II, located on the surface of these lipoproteins. LPL is synthesized by the parenchymal cells of a variety of extrahepatic tissues, most notably in myocytes and adipocytes (1–3). Hepatic lipase (HL), on the other hand, hydrolyzes triglycerides and phospholipids of apoC-II-deficient lipoproteins. HL is synthesized in hepatocytes and functions primarily as an endothelial-bound enzyme within the liver sinusoids (4–6).

LPL and HL belong to an extended gene family that also includes pancreatic lipase (PL), the predominant enzyme required for hydrolysis of dietary triglycerides. These three lipases appear to have evolved from a common primordial gene, based both on homology of the amino acid sequences and on the intron/exon organization of their genes (7, 8). From the proposed lipase phylogenetic tree, it appears that PL diverged relatively early from the ancestral gene, whereas LPL and HL share a more recent root. Thus, these latter two enzymes share, to a larger extent, homologous features, including possible sites of asparagine-linked glycosylation.

Two potential glycosylation sites, located in the amino- and carboxy-terminal domains, are preserved in LPL and HL of all known species (8). Besides these conserved sites, human HL contains two additional glycosylation sites, each preceding the conserved sites by 36 and 35 amino acids, respectively (**Fig. 1A**). The importance of Asn-linked glycosylation for expression of lipase activity has been demonstrated primarily by the action of tunicamycin, which inhibits the cotranslational transfer of oligosaccharide from dolichol-P-P to the nascent polypeptide chain. In the presence of this drug, both LPL and HL

Abbreviations: LPL, lipoprotein lipase; HL, hepatic lipase; PL, pancreatic lipase; PCR, polymerase chain reaction; wt, wild type; PBS, 0.15 M NaCl in 0.1 M sodium phosphate buffer, pH 7.4; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Staph A, crude insoluble protein A from lyophilized *Staphylococcus aureus* cells; TBS, 0.15 M NaCl in 10 mM Tris buffer, pH 7.4; TBS-T, 10 mM Tris buffer, pH 7.4, containing 0.15 M NaCl and 0.1% Tween-20; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride; ER, endoplasmic reticulum.

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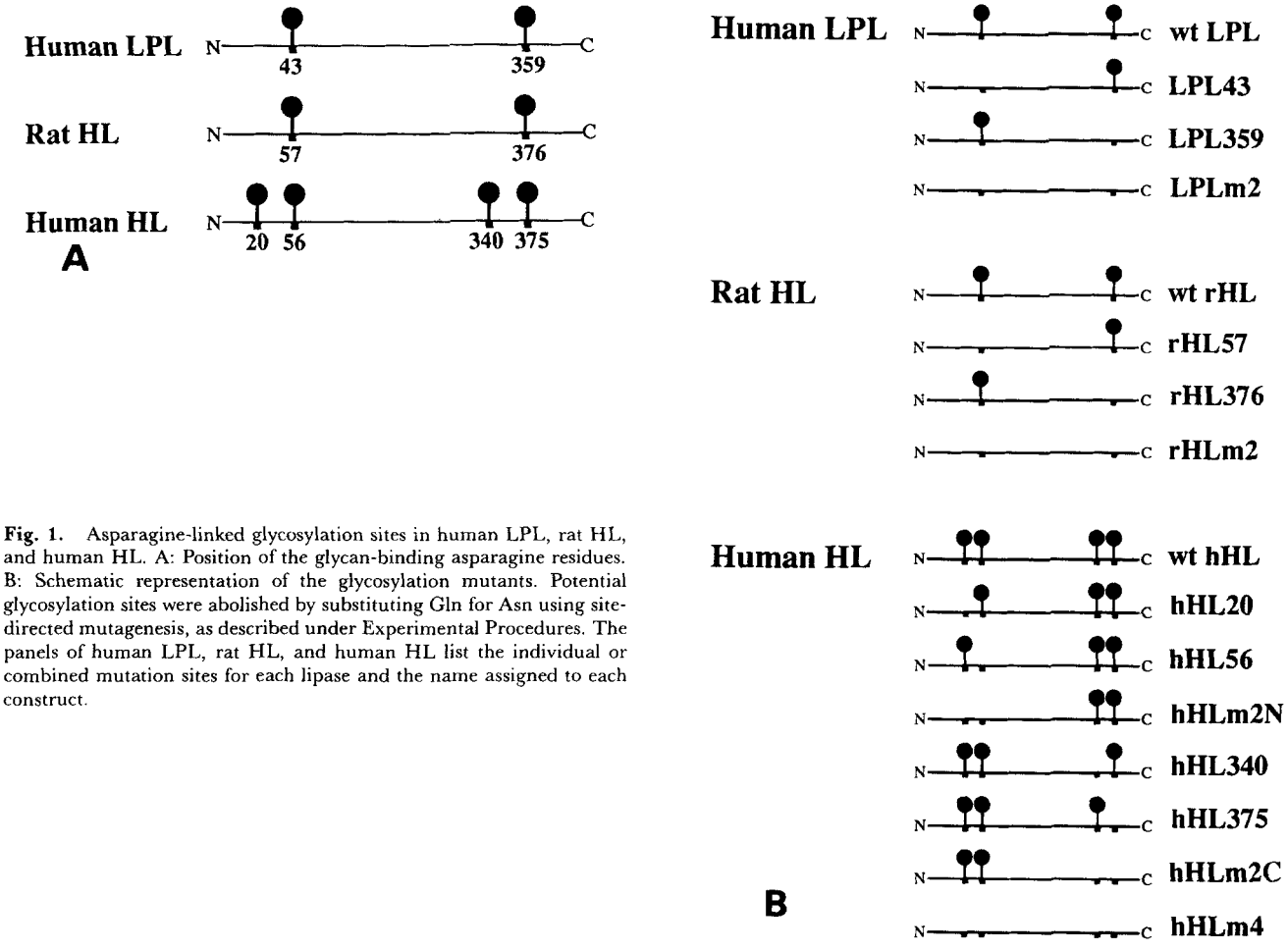

Fig. 1. Asparagine-linked glycosylation sites in human LPL, rat HL, and human HL. A: Position of the glycan-binding asparagine residues. B: Schematic representation of the glycosylation mutants. Potential glycosylation sites were abolished by substituting Gln for Asn using site-directed mutagenesis, as described under Experimental Procedures. The panels of human LPL, rat HL, and human HL list the individual or combined mutation sites for each lipase and the name assigned to each construct.

were shown to be synthesized as inactive enzymes that, in most cases, failed to be secreted (9–13). Incubation of rat adipocytes in glucose-free medium also resulted in unglycosylated LPL that was inactive and not secreted (14). However, the use of tunicamycin and glucose deprivation were indirect methods to demonstrate the importance of glycan chains for lipase activity, as glycosylation of all cellular proteins is inhibited under these conditions. Some of these cellular proteins may be important in the maturation of the lipases to an active form. Furthermore, as lipases contain multiple glycan-binding sites, these conditions were not suitable to evaluate the role of glycosylation at individual sites of the lipase molecule.

To study these individual sites, site-directed mutagenesis has been used to address the importance of glycosylation in human LPL, rat HL, and, most recently, human HL. For human LPL, Semenkovich et al. (15) showed a direct relationship between lack of glycosylation of the N-terminal domain of human LPL (Asn 43) and abolition of LPL catalytic activity expressed in COS cells. For rat HL (16), site-directed mutagenesis was used to abolish either individually or in tandem the two glycosyation sites. These HL mutants, expressed in Xenopus oocytes, showed that deleting one or both glycosylation sites caused the progressive decrease of HL activity in both cells and medium. As the small amount of activity secreted by the double-mutant HL had a correspondingly small amount of mass, it was concluded that glycosylation was not essential for rat HL catalytic activity. Finally, for human HL, Wölle et al. (17) used a CHO expression system and found that deleting the conserved glycosylation site at the N-terminal domain caused the complete abolishment of enzyme activity and secretion.

From the above studies, it was concluded that glycosylation of human HL and LPL, at least at the N-terminal domain, was essential for the expression of a functional enzyme; in contrast, rat HL seemed to differ with regard to this absolute requirement since, even when all glycan chains were removed, a small portion was secreted and catalytically active. We attempt in the present study to resolve these apparent discrepancies by comparing the glycosylation requirements of human LPL, rat HL, and human HL in a uniform expression system. This analysis was done by systematically deleting the glycosylation sites

of each enzyme, either individually or in various combinations, and expressing all mutants in COS cells. We show that these deletions disrupt the normal expression of human LPL, rat HL, and human HL, in a manner dependent on both the position and the number of glycan chains abolished. While the activity of all lipases was affected by the deletion of glycosylation, particularly at the N-terminal domain, the degree of this effect differed between LPL and the two species of HL examined.

EXPERIMENTAL PROCEDURES

Materials

The expression vector pSVL was purchased from Pharmacia. Vent polymerase was from New England BioLabs. *Bam*H I and *Xho* I were from Promega. COS-7 cells were originally purchased from the American Culture Collection. The blocking solution for HL Western blotting and polyvinylidene difluoride (PVDF) membranes were from United States Biochemical. Heparin, Triton X-100, Tween-20, protein A Sepharose, and *Staphylococcus aureus* were from Sigma. Bovine milk LPL was a generous gift from Dr. John Goers, San-Luis Obispo, CA. Streptavidin-horseradish peroxidase was purchased from Gibco-BRL.

Mutagenesis

Oligonucleotide-directed mutagenesis was used to change the Asn codons in the potential glycosylation sites to glutamine codons. In each case, the mutant codon was centered in a 21- to 42-nucleotide oligomer that was otherwise consistent with the original cDNA sequence (**Table 1**). All mutations were verified by dideoxynucleotide se-

TABLE 1. Oligonucleotides for site-directed mutagenesis

Mutant[a]	Mutation Site													
LPL														
	373			(AAT)										
LPL43	TGT	CAT	TTC	CAG	CAC	AGC	AGC	AA						
	Cys-40	His	Phe	Gln	His	Ser	Ser							
	1321			(AAT)										
LPL359	GTT	TCC	ACA	CAG	AAG	ACC	TAC	TC						
	Val-356	Ser	Thr	Gln	Lys	Thr	Tyr							
Rat HL														
	242			(AAC)										
rHL57	GT	GGC	TTC	CAG	AGC	TCC	CAT	CC						
		Gly-55	Phe	Gln	Ser	Ser	His							
	1199			(AAT)										
rHL376	TT	ACC	AGC	CAG	AAA	ACC	TAT	TCC						
	Thr-374		Ser	Gln	Lys	Thr	Tyr	Ser						
Human HL														
	120			(AAC)										
hHL20	CT	GTT	GAA	ACA	CAA	AAA	ACG	CTG						
		Val-17	Glu	Thr	Gln	Lys	Thr	Leu						
	229			(AAC)										
hHL56	G	TGC	GGC	TTC	CAA	TCC	TCC	CTG	C					
		Cys-53	Gly	Phe	Gln	Ser	Ser	Leu						
	1081			(AAC)										
hHL340	C	CAG	TTC	ATC	CAA	CAA	ACT	GAG	AC					
		Gln-337	Phe	Ile	Gln	Gln	Thr	Glu						
	1184			(AAT)										
hHL375	5'- GGA	ATT	GCT	AGT	CAA	AAA	ACG	TAT	TCC-3'					
hHL375-rev.	3'- CCT	TAA	CGA	TCA	GTT	TTT	TGC	ATA	AGG	AAA	GAA	TAG	TGC	GAC-5'
	Gly-371	Ile	Ala	Ser	Gln	Lys	Thr	Tyr	Ser	Phe	Leu	Ile	Thr	Leu
Additional oligonucleotides[b]														
pSVL														
	1458													
pSVL-NC	5'- TCT	AGG	CCT	GTA	CGG	AAG	TGT-3'							
	1572													
pSVL-CN	3'- TCA	AAC	CTG	TTT	GGT	GTT	GAT-5'							

Human LPL, rat HL, and human HL were subjected to site-directed mutagenesis as described under Experimental Procedures. For each lipase mutation, the putative glycan-binding Asn codon was changed to a glutamine codon.

[a] Sequences are according to refs. 19 (LPL), 20 (rHL), 26 (hHL) and 24 (pSVL).

[b] Oligonucleotides for the pSVL plasmid were used as primers in the PCR reactions to generate mutant hHL375.

quencing (18). Figure 1B shows the positions of the glycosylation sites that were mutated in human LPL, rat HL, and human HL.

LPL and rat HL. Mutagenesis of human LPL (19) and rat HL (20) was carried out as described (16) using phosphorothioate nucleotides (21–23) and instructions from Amersham. The constructs were transferred into the polylinker region of the pSVL expression vector (24).

Human HL. For mutations of human HL (25, 26), the gapped duplex method and the pMca5-8 vectors described by Stanssens et al. (27) were used to create all constructs except hHL375. The 375 mutant was generated by sequential polymerase chain reactions (PCR) (28) using VENT polymerase. In the first stage, using wild-type hHL cDNA in pSVL as template, two overlapping PCR products were generated. For the first product, the forward orientation of the mutation oligonucleotide was paired with pSVL-CN, a 3' to 5' primer homologous to pSVL sequences located outside the C-terminal end of the polylinker region (Table 1). For the second PCR product, the reverse complement of the mutation oligonucleotide was paired with pSVL-NC, a primer homologous to pSVL sequences just outside the N-terminal end of the polylinker (Table 1). The full-length hHL375 cDNA was obtained in a third PCR reaction, using as template the two overlapping products and the same flanking primers used in the first stage reactions. The final product was excised with *Xho* I and *Bam*H I and recloned into pSVL. The complete nucleotide sequence of hHL374 was verified by sequencing (18).

In vitro expression and lipase activity assay

COS-7 cells were grown in DMEM containing 10% calf serum. For transfection with each of the constructs, duplicate aliquots containing each 1.7×10^7 cells were centrifuged and resuspended in 0.8 ml electroporation buffer (PBS containing 20 mM HEPES, pH 7.4). Plasmid DNA (40 μg) was added to each aliquot and the mixtures were kept on ice for 10 min. For electroporation, the suspensions were transferred to Gene Pulser cuvettes (0.4 cm path length) and exposed to a single voltage pulse (0.33 kV, 960 μF) using a Bio-Rad Gene Pulser Transfection Apparatus. After electroporation, the cell suspensions were kept on ice for 5–10 min and then plated into 60-mm tissue culture plates. The media were changed after 4 h and again after 24 h, at which time heparin was added (10 U/ml) to half of the plates in each group. After an additional overnight incubation, media were collected and the cells were harvested.

Cell pellets were homogenized in 200 μl extraction buffer (50 mM ammonia buffer, pH 8.0, containing 0.125% Triton X-100). After a 5-min centrifugation, the supernatants were assayed immediately for LPL or HL enzymatic activity (5, 29). One millunit of enzyme activity represents the release of 1 nmol of free fatty acid/min.

Antibodies

Bovine milk LPL was used to innoculate chicken hens (30). IgG was purified from the hens' egg yolks using the method of Loeken and Roth (31). Typically, 10 egg yolks yielded approximately 450 mg IgG. Further purification by affinity chromatography on LPL-Sepharose (30) yielded 3 mg of anti-LPL IgG.

Antibodies against rat HL were prepared in rabbits as described previously (5).

Antibodies against human HL were raised in rabbits against three fusion proteins. These fusion proteins, prepared according to Strebel et al. (32), contained an 11 kDa fragment of MS-2 polymerase linked to human HL peptides spanning amino acids −8 to 175, 149 to 328, and 278 to 475, respectively.

Immunoprecipitations

An immunoaffinity complex between Staph A and antibodies against each lipase was prepared (33, 34). The cross-linked immunomatrix was washed twice with borate buffer (0.2 M Na borate, pH 8.0) containing 0.5 M NaCl, once with 0.2 M glycine buffer, pH 2.7, containing 0.5% Triton X-100, and two additional washes with borate buffer. The immunomatrix was stored at −80°C in PBS containing 0.5% Na azide.

Prior to HL immunoprecipitation, samples of cell extracts or media were denatured by addition of SDS to 0.5% and boiling for 2 min. After denaturation, these samples were mixed with a concentrated lysis buffer to give a final concentration of: 0.1 M Tris, pH 7.5, 0.15 M NaCl, 1 mM EDTA, 2% Triton X-100, 0.5% N-lauroyl-sarcosine, 0.1% SDS, and the following protease inhibitors: 50 μg/ml soybean trypsin inhibitor, 20 kallikrein units/ml aprotinin, 157 μg/ml benzamidine, 4 μg/ml leupeptin, and 1 μg/ml pepstatin. Samples for LPL immunoprecipitation were prepared in a similar manner, except that denaturation was omitted and the NaCl concentration was increased to 1.0 M. After addition of the appropriate immunomatrix, the samples were incubated at 4°C for 48 h. The immunomatrix was then washed once with 0.5 M LiCl in Tris 0.1 M, pH 7.5, twice with lysis buffer, and once with 50 mM sodium phosphate buffer, pH 5.9, containing 0.1% N-lauroyl-sarcosine. The antigen was released from the immunocomplex by boiling for 2 min in sodium phosphate buffer (50 mM, pH 5.9) containing 0.5% SDS and 20% glycerol. SDS-polyacrylamide gel electrophoresis (PAGE) was carried out as previously described (33).

Western blotting and chemiluminescence detection

Biotinylated antibodies were prepared according to the protocol outlined by Harlow and Lane (35). After PAGE, the gels were blotted at 200 mA for 60 min onto PVDF membranes. Blotted membranes were incubated overnight in blocking buffer: for LPL blots, the blocking

buffer was 3% casein hydrolysate in TBS containing 0.1% Tween-20 (TBS-T); for HL blots, the blocking solution was prepared using the reagents supplied in Protein Images™ Western Blotting detection kit. After blocking, the membranes were incubated overnight at 4°C with the respective biotinylated antibodies (0.3 μg/ml biotinylated anti-LPL or 1 μg/ml biotinylated anti-HL). After washing three times for 5 min with TBS-T, membranes were incubated with streptavidin-horseradish peroxidase diluted 1:2500 in TBS-T for 30 min. The blots were then washed 3 times with TBS containing 0.3% Tween-20, followed by 3 washes with TBS-T, and coated for 60 sec with the chemiluminescence solution (reagents and instructions from Amersham, ECL Western Blotting detection kit). The membranes were exposed to Kodak X-Omat AR film for 10–40 sec.

Scanning densitometry and quantitation of lipase mass

Densitometric scanning of the bands obtained by chemiluminescence was carried out with the aid of an LKB Laser Densitometer. Integration of the scanned peaks was performed using the Macintosh Hoefer Gel Scanner software. As shown by immunoprecipitating increasing amounts of LPL or HL with fixed amounts of immunomatrix (**Fig. 2**), the peak area was directly proportional to the quantity of lipase protein up to the point of antibody saturation.

Other methods

Statistical significances were determined by the unpaired two-tailed *t*-test, using the StatView II software for the Macintosh. Significance was assigned at $P \leq 0.01$. Specific activity was calculated as the ratio between the activity of the sample subjected to Western blotting and the mass detected by densitometric scanning. The ratio obtained for wild type was designated as 1.0, and the specific activity of the associated mutants was expressed relative to this value.

RESULTS

This study addresses the role of glycosylation at individual or multiple sites in LPL and HL, two members of the lipase gene family. To accomplish this objective, panels of human LPL, rat HL, and human HL mutants lacking specific glycosylation sites were constructed as shown in Fig. 1B, and transiently expressed in COS cells. Transfected cells were incubated with or without heparin in the medium. This was done since extracellular and intracellular lipase levels are maximal in the presence and absence of heparin, respectively (4, 36).

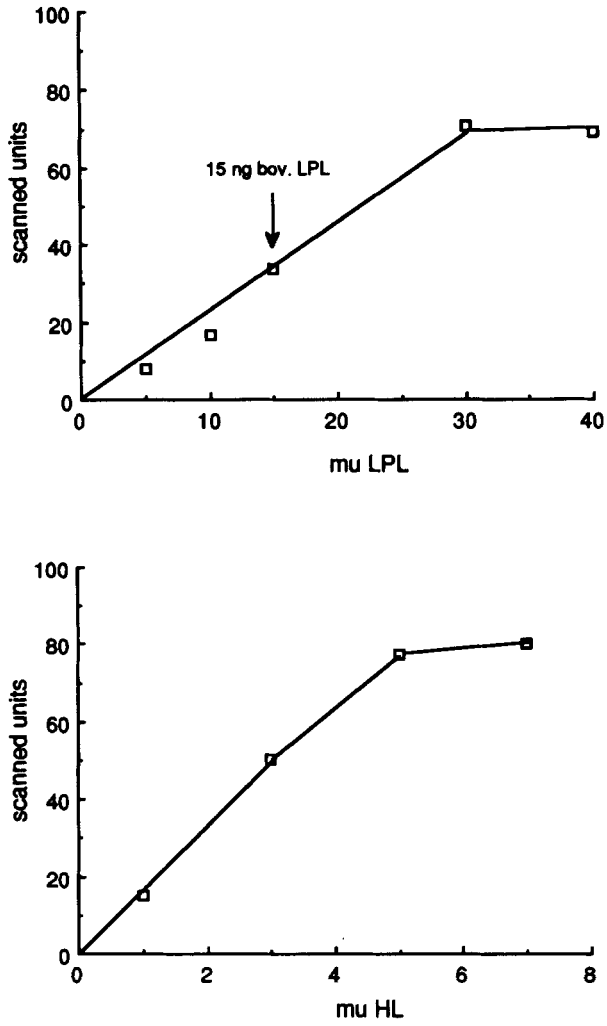

Fig. 2. Relationship between lipase mass and intensity of chemiluminescence. Increasing amounts of heparin-containing medium from plates transfected with wild-type LPL and human HL were immunoprecipitated with 40 μl of the respective immunomatrix. After Western blotting and detection by chemiluminescence, the intensity of the bands was measured by scanning densitometry, as described under Experimental Procedures. In the LPL titration experiment, a sample of 15 ng purified bovine milk LPL was also immunoprecipitated. Note that the scanned intensity of this LPL sample corresponds to the intensity that would be obtained with 15 mu human LPL, indicating that human LPL specific activity is about 1 mu/ng.

LPL

Individual mutations at asparagine residues 43 and 359 of human LPL cDNA were generated (LPL43 and LPL359). In addition, we investigated the expression of a construct where both glycosylation sites of LPL were eliminated (LPLm2). As shown in **Fig. 3,** LPL359 was as active as the wild-type, both in the cells and media. As expected, heparin increased enzyme activity in the media

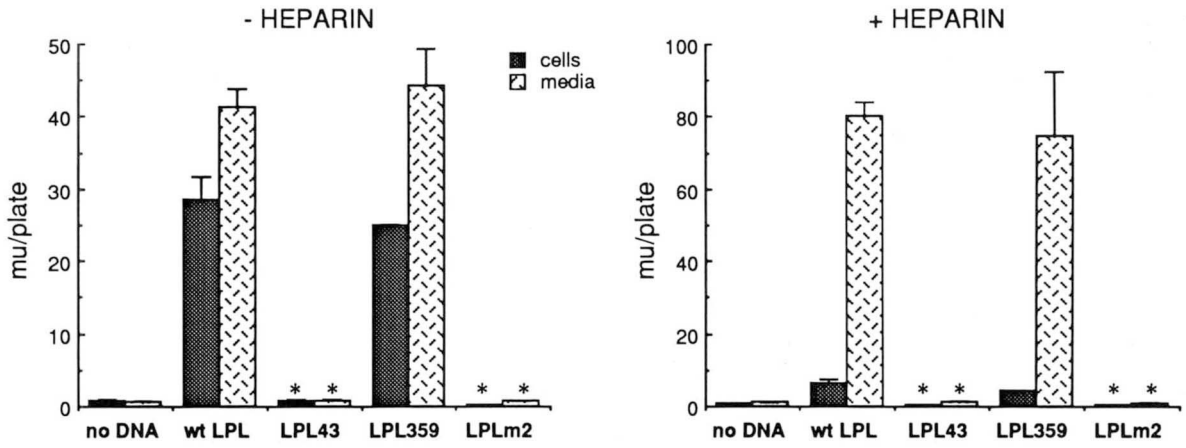

Fig. 3. LPL activity in wild type and glycosylation mutants. Twenty-four hours after transfection, media were changed and heparin (10 U/ml) was added to some plates. After 18 h, cells and media were harvested, and the cell pellets were homogenized as described under Experimental Procedures. Values represent the mean ± SD of four plates. *Activity levels are not significantly different than those of untransfected cells ($P > 0.05$).

about twofold. In contrast, under no condition did LPL43 and LPLm2 express any significant activity compared to untransfected cells.

LPL protein was detected after SDS PAGE and immunoblotting. In this manner, both glycan utilization and relative mass could be determined. **Figure 4** indicates that in the single mutants, LPL protein migrated at increased rates relative to wt LPL (lanes 1–3) and at an even higher rate in the double mutant (lanes 4, 6). Thus, both the N-terminal and C-terminal glycosylation sites in human LPL are utilized. Intracellularly, LPL mass was present regardless of the glycosylation state, albeit at reduced levels in the unglycosylated LPLm2 (lane 4). This decreased mass could indicate instability of the unglycosylated form of LPL. Secretion of LPL protein from the catalytically active wt LPL and LPL359 was comparable (lanes 8, 10). On the other hand, catalytically inactive LPL was retained intracellularly, as no mass could be detected in the media of the inactive mutants LPL43 and LPLm2 (lanes 9, 11).

In summary, LPL lacking the glycosylation site at the N-terminal domain (LPL43), as well as unglycosylated LPL (LPLm2), was completely inactive and not secreted. In contrast, a similar mutation at the C-terminal domain (LPL359) had no significant effect on enzyme expression.

Rat HL

Rat hepatic lipase also has two glycosylation sites, at positions homologous to those of LPL. The rat HL panel (Fig. 1B) included constructs lacking a single site (rHL57 and rHL376), and a construct lacking both sites (rHLm2). **Figure 5** shows the expression of wild-type rat HL and the associated mutants. Similar to LPL, removal of the glycan chain at the C-terminal domain (rHL376) had no effect on enzyme expression, while glycan removal at the N-terminal domain (rHL57) resulted in a marked decrease in enzyme activity. The decrease occurred both intracellularly and in the medium, either in the presence or in the absence of heparin. However, while deletion of the homologous N-terminal glycosylation site in LPL (LPL43) resulted in complete loss of catalytic activity, rHL57 still expressed about 20% of wild-type activity. Regarding the double mutant (rHLm2), in most cases we were unable to detect any activity significantly higher

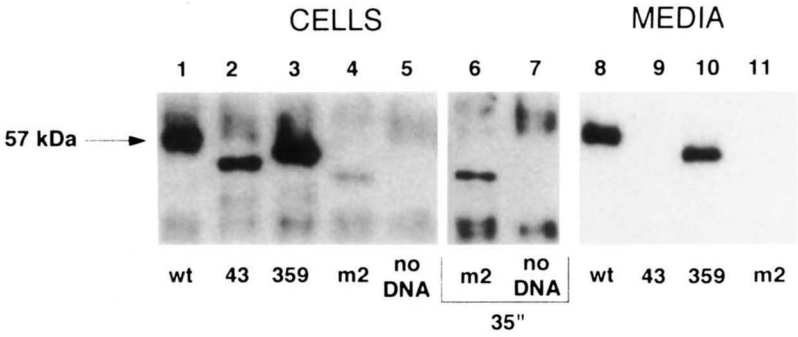

Fig. 4. LPL mass in cells and media. Cell lysates representing 0.5 mg of total protein and 1 ml of media were immunoprecipitated with LPL antibody as described under Experimental Procedures. After PAGE and Western blotting, the protein bands were detected by chemiluminescence. Membranes were exposed to film for 10 sec, except for LPLm2, where a longer exposure time (35") was necessary.

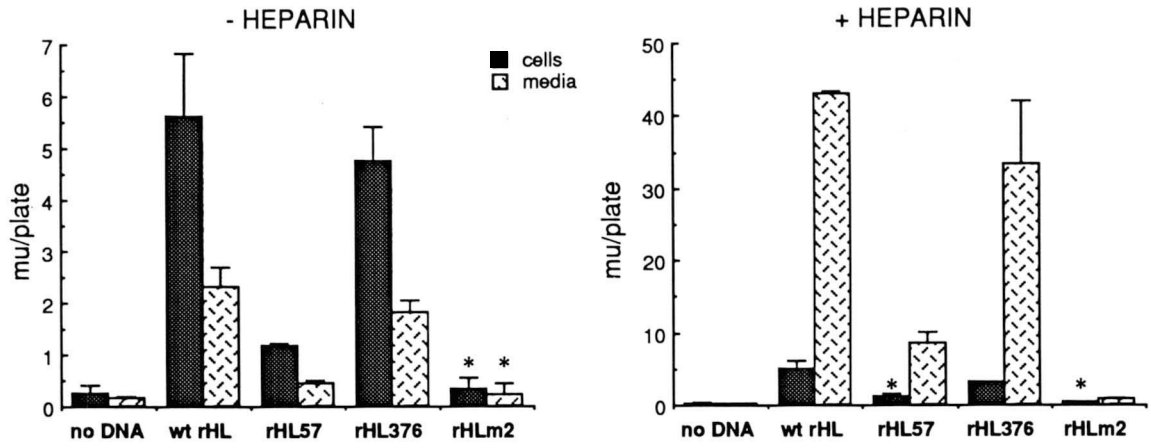

Fig. 5. Rat HL activity in wild type and glycosylation mutants. Cells were transfected and maintained as described in the legend to Fig. 3. Prior to harvesting, the cells were incubated for 18 h in the presence or absence of heparin. Values represent the mean ± SD of four plates. *Activity levels are not significantly different than those of untransfected cells ($P > 0.05$); all other values are significant to the level of $P < 0.005$.

than the background levels of untransfected cells. Nevertheless, we did detect in heparinized medium a very small increase in activity above background levels ($P = 0.005$).

Figure 6 shows the presence of HL mass in cells and media of wild-type rHL and the glycosylation mutants. The increased mobility of the single mutants (lanes 3, 4) and the further increased mobility of the double mutant (lane 5) indicates that both potential glycosylation sites in rat HL are utilized. Intracellularly, comparable amounts of HL mass were expressed by all constructs, regardless of activity levels (lanes 2–5). Besides the main band migrating at the 57 kDa region, a degradation product of HL migrating within the 35 kDa region is also visible. This fragment exhibits a lower molecular weight in the mutants lacking glycan at the N-terminal domain (rHL57 and rHLm2) compared to wild-type rHL and rHL376, where glycosylation at this site is intact. This suggests that the 35 kDa HL product originates from the N-terminal domain of the molecule.

In contrast to the cells, the media contained levels of HL protein proportional to the levels of secreted enzyme activity (Fig. 6, lanes 6–10). Specifically, while the amount of HL protein was similar in rHL376 and wild-type rHL, only small amounts were secreted by rHL57 (lanes 6–8). At standard exposure times, secreted protein from rHLm2 was hardly perceptible; after a longer exposure, a faint band representing unglycosylated HL became apparent (lanes 9 and 10).

Human HL

Besides the two conserved glycosylation sites, human HL contains two additional non-conserved glycosylation sites (Fig. 1A). Thus, four hHL constructs, with mutations abolishing each individual site, were generated. In addition, mutants lacking a combination of the first two or last two glycosylation sites (hHLm2N, hHLm2C, respectively) were constructed. The rationale was to create unglycosylated hHL mutants at either the N-terminal or the C-terminal domain, analogous to the mutants lacking glycosylation sites at these respective domains in human LPL or rat HL (Fig. 1B). Finally, a quadruple mutant (hHLm4) encoding hHL with no glycosylation sites was also investigated.

Figure 7 shows the expression of HL activity in each of

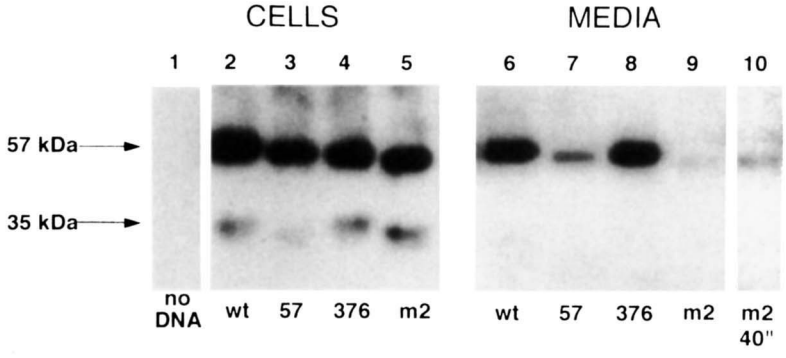

Fig. 6. Rat HL mass in cells and media. Cell lysates representing 0.5 mg of total protein and 1 ml of media were immunoprecipitated with HL antibody. Immunoprecipitates were subjected to PAGE and Western blotting as described under Experimental Procedures. Membranes were exposed to film for 10 sec, and an increased exposure (40") is shown for the medium sample of rHLm2.

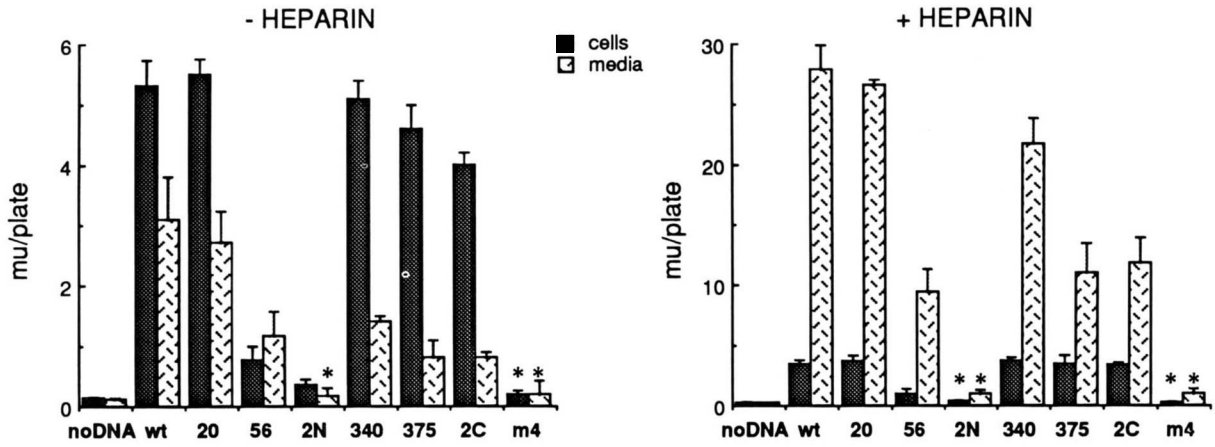

Fig. 7. Human HL activity in wild type and glycosylation mutants. Human HL constructs were transfected into COS cells, and the cells were incubated in the absence or presence of heparin as described in the legend to Fig. 3. Values represent means ± SD from four plates. *Activity levels are not significantly different than those of untransfected cells ($P > 0.05$); all other values were significant to the level of $P < 0.005$.

these constructs, in the absence or presence of heparin. Mutant hHL20, lacking the unconserved glycosylation site at the N-terminal domain, displayed the same catalytic activity as wild-type hHL. In contrast, the conserved N-terminal mutant hHL56 showed approximately a 70% decrease in lipase activity, both intracellularly and in the medium. This reduction was similar to the decreased HL expression in the homologous rat mutant rHL57. Unexpectedly, removal of both glycosylation chains at the amino-terminal domain of human HL (hHLm2N) nearly abolished enzyme activity. Only a very small amount of intracellular activity in cells was detected when incubated in the absence of heparin ($P < 0.005$); no activity above background could be detected either in cells grown in the presence of heparin, or in the medium under any condition. This suggests the occurrence of a synergistic effect when both glycosylation sites of this domain are abolished.

The HL mutants of the C-terminal domain (hHL340, hHL375, and the double mutant hHL2C) displayed intracellular HL activities similar to the wild type (Fig. 7). However, in almost all cases, HL secretion from these mutants was impaired. In the absence of heparin, HL secretion was lower in all three C-terminal mutants. In the presence of heparin, secretion from hHL375 and hHLm2C dropped by 60% and only hHL340 contained activity levels close to those of wild type. The completely unglycosylated mutant (hHLm4) displayed no detectable activity, either in the cells or medium.

HL mass expressed by the panel of hHL mutants is shown in **Fig. 8** and **Fig. 9.** Intracellularly (Fig. 8), wild-type hHL and most mutants displayed two prominent bands: intact HL in the 57 kDa region and a principal degradation product found in the 35 kDa region (lanes 2–4 and 6–8); the two inactive mutants, hHLm2N and hHLm4, were present primarily as the degradation product, with a small amount of mass in the region corresponding to the intact protein (lanes 5, 9). Similar to rat HL, the degradation product from the N-terminal domain mutants (lanes 3–5) displayed increased mobility compared to either wild-type hHL (lane 2) or to the C-terminal mutants (lanes 6–8). This again indicates that the 35 kDa degradation product is derived from the N-

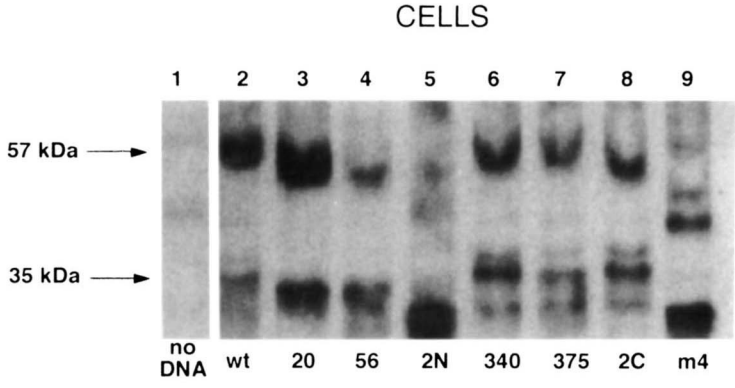

Fig. 8. Human HL mass in cell lysates from wild-type and glycosylation mutants. Lysates were processed as described under Experimental Procedures and in the legend to Fig. 6.

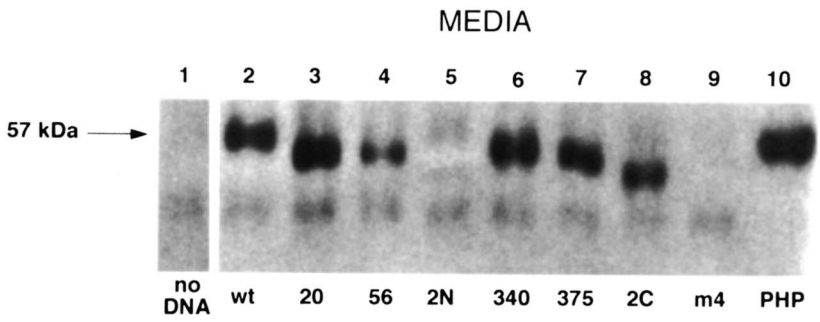

Fig. 9. Human HL mass in the media. Equal amounts of media (1 ml) from each group were subjected to immunoprecipitation and Western blotting as described under Experimental Procedures. In addition, a sample of human post-heparin plasma purified by heparin-Sepharose chromatography (equivalent to 5 mu HL activity) was also included (PHP, lane 10).

terminal domain of the molecule.

Figure 9 shows the pattern of human HL mass secreted to the media. All four single mutants displayed a reduced molecular weight compared to wild type; additionally, a further decrease is seen in the molecular weight of the double mutant hHLm2C. This indicates that human HL, like the other lipases investigated, utilizes all potential glycosylation sites. Comparison of the N-terminal domain mutants with wild-type HL shows that, while hHL20 secreted normal amounts of mass, only a diminished amount was secreted from the partially active mutant hHL56 and virtually no mass was detected in the media of the inactive hHLm2N (lanes 2–5). The mutants of the C-terminal domain secreted HL mass proportional to the detected levels of activity, while the unglycosylated HL mutant secreted no detectable HL mass (lanes 6–9).

Lipase specific activity

The amount of lipase protein was estimated by densitometric scanning of the Western blots shown in Figs. 4, 6, and 8 and used to calculate specific activities. The specific activity of the mutants was normalized to wild-type values which were assigned a value of 1.0. As shown in **Fig. 10A,** abolishing the conserved glycosylation sites at the N-terminal domain resulted in the intracellular expression of completely inactive enzyme (LPL43), or lipase protein that had lost between 75–85% of activity (rHL57 and hHL56, respectively). As opposed to this marked reduction of intracellular HL specific activity, the small amounts of secreted rHL57 and hHL56 had specific activities comparable to wild type (Fig. 10B).

In contrast to the effect of deglycosylation at the N-

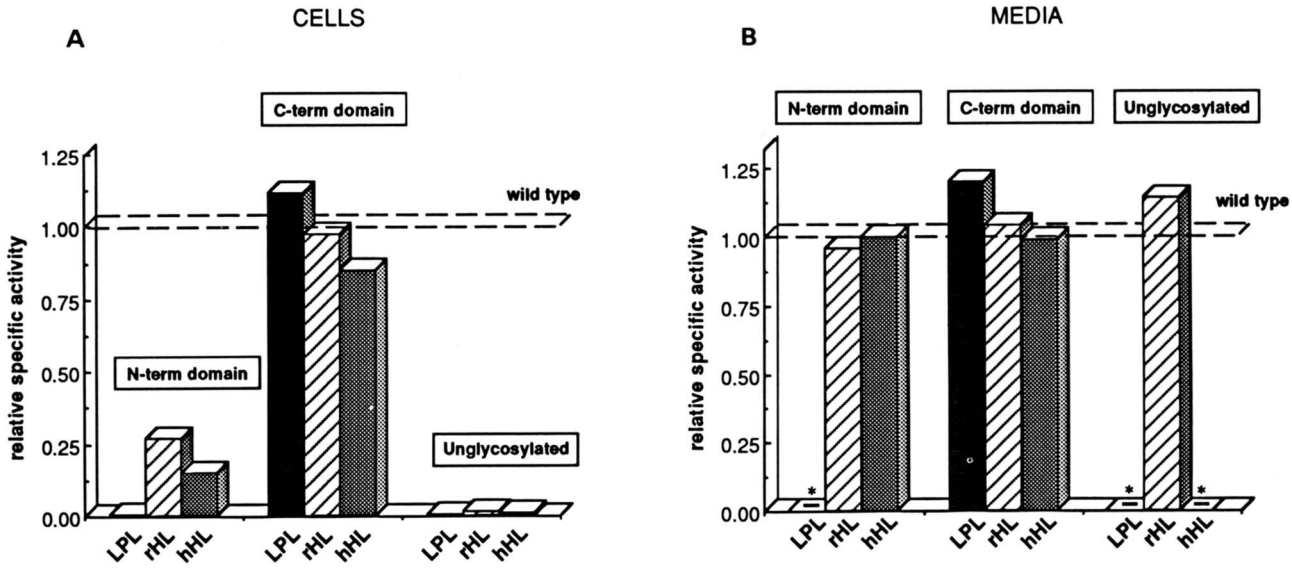

Fig. 10. Specific activity of lipase glycosylation mutants. After Western blotting and detection by chemiluminescence, the lipase mass was assessed quantitatively by densitometric scanning. Specific activity was calculated as the ratio between milliunits of immunoprecipitated sample and the arbitrary mass units obtained by densitometry. The ratio obtained for wild type was designated as 1.0, and the specific activity of the associated mutants was expressed relative to this value. A: Relative specific activity of intracellular LPL and HL glycosylation mutants. The figure shows the specific activity of all LPL and rat HL mutants. For the individual mutants of human HL, only the conserved sites are included (hHL56 and hHL375). B: Relative specific activity of secreted LPL and HL glycosylation mutants. Again, the figure shows the specific activity of all LPL and rat HL mutants. For the individual mutants of human HL, only the conserved sites are included (hHL56 and hHL375). *The short horizontal lines indicate that in these cases the calculation of specific activity is irrelevant, as no secreted lipase could be detected.

terminal domain, lipase molecules lacking glycan at the C-terminal domain had specific activities comparable to the wild type. However, deglycosylation of both domains resulted in the expression of virtually inactive lipases (Fig. 10A). Among these three unglycosylated enzymes, rHLm2 was the only lipase exhibiting a barely perceptible level of activity (2%) in heparinized medium (Fig. 5). As this small amount of activity corresponded to a proportionally small amount of mass, the specific activity for secreted rHLm2 was comparable to wild type (Fig. 10B). Thus, unglycosylated rat HL, although primarily inactive and retained intracellularly, still has the ability to form a small number of active HL molecules.

As human HL has two additional (unconserved) glycosylation sites at the N- and C-terminal domains, the intracellular specific activities of the hHL mutants are separately shown in **Fig. 11.** It is clear from this figure that deleting the unconserved glycosylation site at the N-terminal domain (hHL20) does not decrease the specific activity. As discussed above, deleting the conserved site at the N-terminal domain (hHL56) resulted in the loss of 85% specific activity; however, when both the conserved and unconserved sites were removed at this domain (hHLm2N), the enzyme became essentially inactive (specific activity = 0.04). In contrast to the N-terminal mutants, specific activities remained unchanged in all C-terminal mutants, regardless of the position or number of sites deleted. In the media, specific activity of all secreted hHL mutants (i.e., all mutants except hHLm2N and hHLm4) was unaffected (data not shown).

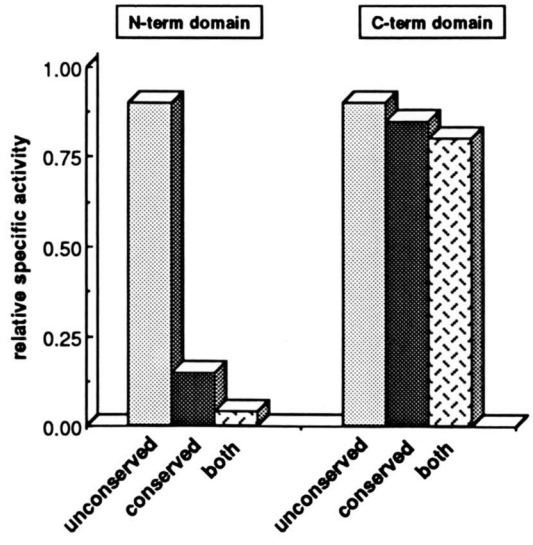

Fig. 11. Relative specific activity of intracellular human HL glycosylation mutants. Shown are the specific activities of the human glycosylation mutants at the individual (conserved and unconserved) sites at the N-terminal and C-terminal domains (hHL20, hHL56 and hHL340, hHL375, respectively). In addition, the mutants lacking both sites at each domain are included (hHLm2N and hHLm2C).

DISCUSSION

This study addresses the importance of asparagine-linked glycosylation in two members of the lipase gene family, LPL and HL. Human LPL and rat HL have two homologous Asn-linked sites in highly conserved regions; human HL contains two additional sites in less conserved regions. The third member of the lipase gene family, PL, presumably diverged earlier than LPL and HL during evolution and does not share the conserved glycosylation sites. Instead, there is a single potential Asn-linked glycosylation site in human and porcine PL (37, 38), and one site at a different location in canine PL (39). Equine PL has no glycosylation sites at all (40). Thus, the conservation of glycosylation sites in LPL and HL, but not in PL, would imply that glycosylation is essential for the expression of these two members of the lipase gene family. Indeed, our study indicates that glycosylation overall is important for full expression of these two lipases; however, it also shows that removal of individual glycosylation sites (specifically at the N-terminal domain) affects the expression of LPL and HL to different degrees.

Mutants lacking individual glycosylation sites

N-terminal domain. The primary sequence homology and the deduced secondary structure among LPL, HL, and PL strongly suggest that the three molecules exhibit common folding patterns that result in similar three-dimensional structures (41, 42). Based on the known crystallographic structure of human PL (43), the approximate location of the glycosylation sites in LPL and HL can thus be inferred. From their deduced placement, it is clear that none of these glycosylation sites are in close proximity to the catalytic triad, nor are they located in regions that have been postulated to participate actively in enzyme function (44–47). Nevertheless, as shown in this study, abolishing the conserved glycan chain at the N-terminal domain resulted in a dramatic loss of enzyme activity and secretion; thus, no LPL activity was detected in LPL43 while some (approximately 25%) HL activity remained in rHL57 and hHL56 (Figs. 3, 5, 7). Intracellularly, lipase mass was found in amounts comparable to the wild-type controls, resulting in a either a complete (LPL) or 75–85% (HL) reduction of specific activity (Fig. 10A). The reduction of HL specific activity could result from two possibilities: *i)* each mutant molecule expresses only partial activity; or *ii)* 15–25% of the lipase molecules are fully active, and the remaining HL molecules are completely inactive. According to the first possibility, secreted HL should exhibit a low specific activity, similar to the intracellular value. This possibility was ruled out, since the specific activity of secreted rHL57 and hHL56 was found to be as high as wild-type values (Fig. 10B). Thus, 15–25% of rHL57 and hHL56 appear to attain a form that is similar to wild type, both in activity and in ability

to be secreted; it follows that the remaining molecules are retained intracellularly in a completely inactive form. Wölle et al. (17) also found that removal of the glycan chain at Asn 56 of human HL resulted in the formation of inactive enzyme that was retained intracellularly. However, their study showed that hHL56 was completely inactive, while in our experiments, we were still able to detect a portion of HL mutant molecules that were active and secreted. Thus, according to our data, glycan removal at the conserved site at the N-terminal domain had a similar effect on both human and rat HL. At this point, the reason for the discrepancies between the work of Wölle et al. (17) and the present study is unclear.

The inactive form of LPL and HL lacking glycan at the N-terminal conserved site could derive from the failure of the nascent polypeptide to fold correctly. Support for this possibility can be found in multiple studies establishing the role of glycosylation as an important factor in proper protein folding (for reviews, see refs. 48, 49). Furthermore, incorrect folding affects the intracellular transport of glycoproteins and ultimately their secretion, since malfolded proteins often do not progress beyond the ER (49, 50). Indeed, inactive LPL and HL in mutants lacking the conserved N-terminal glycosylation site were retained intracellularly. Based on the three-dimensional structure of pancreatic lipase, Derewenda and Cambillau (41) have provided a mechanistic basis for the proposed relationship between lack of glycosylation at the conserved site of the N-terminal domain and improper lipase folding. The authors noted that the glycan-binding site of LPL Asn 43 is closely preceded by the first disulfide bridge of the molecule. Moreover, a Phe residue, located between the glycan-binding Asn residue and the first disulfide bridge, is part of an internal hydrophobic constellation. The authors suggest that in the absence of carbohydrate at this site of LPL, neither the hydrophobic core nor the disulfide bonds are formed. A similar mechanistic argument could explain the inhibition of activity caused by glycan absence at the homologous site in HL, since the first disulfide bridge is conserved in all LPL and HL species and the Phe residue is conserved in all members of the lipase family. Consistent with this argument is the fact that pancreatic lipase lacks both the conserved N-terminal domain glycosylation site and the preceding disulfide bridge, underscoring the importance of glycan presence at this site in LPL and HL, but not in PL.

As mentioned above, while LPL43 was completely inactive, a portion of HL molecules with normal specific activity was expressed in the homologous HL mutants. This may suggest that, unlike LPL, the three-dimensional structure of HL resulting in a catalytically active form is not as strictly constrained; thus, perhaps a fraction of rHL57 and hHL56 assumes a conformation appropriate for full expression and secretion of enzymatic activity. This increased flexibility of the HL molecule to assume an active conformation is implied by the less stringent conservation of the HL primary structure among different species as compared to LPL. For instance, Hide, Chan, and Li (8) calculated a 23.6% divergence between rat and human HL, but only a 2.2% divergence between rat and human LPL.

In addition to the conserved N-terminal glycosylation site at Asn 56, human HL is glycosylated at Asn 20, a non-conserved site. Removal of this glycan has no effect on specific activity (Fig. 11), perhaps because Asn 20 is located well ahead of the first disulfide bridge spanning hHL Cys 40–Cys 53.

C-terminal domain. Based on the crystallographic structure of human PL, the glycoslation sites at the C-terminal domain of HL and LPL appear to be located on external loops, in regions that do not appear to be directly involved in any catalytic interactions (41). Indeed, although these glycosylation sites are normally utilized in the lipases studied here, their absence does not significantly affect enzyme specific activity (Figs. 10, 11). However, it does appear that removal of the glycan chains at the C-terminal domain of human HL can affect enzyme secretion (Fig. 7). The reason for the decreased HL secretion from these mutants is not known. It is possible that the absence of the polar glycan chain increases the hydrophobicity of the lipase molecule, impeding its otherwise efficient transport to the cell surface with the aqueous bulk flow (51).

Mutants lacking multiple glycosylation sites

With one exception (hHLm2C), mutants lacking multiple glycosylation sites had the essential glycosylation site at the N-terminal domain removed in tandem with a nonessential site. LPLm2 was completely inactive and not secreted, as expected from the absolute requirement of the glycosylation site at the N-terminal domain (Fig. 3). Similar to LPLm2, the concurrent removal of one (or more) nonessential sites in rat and human HL caused the near abolishment of HL expression. This was unexpected, since removal of the essential site (rHL57, hHL56) still resulted in the expression of approximately 25% activity. This combined effect could be explained by the function of Asn-linked sugars on overall protein solubility. Oligosaccharide chains, through their hydrophilic nature, may render the folding intermediates of nascent polypeptide chains more soluble and prevent irreversible aggregation (49, 52).

With respect to the lipase protein, unglycosylated rat HL (rHLm2) was retained intracellularly at levels comparable to the active form (Fig. 6), while unglycosylated LPL (LPLm2) was found in reduced amounts (Fig. 4). In human HL, the inactive mutants lacking two or four glycosylation sites (hHLm2N and hHLm4) exhibited increased amounts of lower molecular weight products (Fig. 7). Again, these findings are in agreement with the general concept that without oligosaccharides, proteins tend to misfold, aggregate, and often get degraded

without transport from the ER to the Golgi complex (48, 49, 53, 54).

A very small amount (2%) of the unglycosylated rat HL was catalytically active and secreted (Figs. 5, 10B). This finding is in full agreement with that of Stahnke et al. (16), who also reported that only a small portion (3%) of unglycosylated rat HL was secreted and had a normal specific activity. In this respect, our data support the conclusion of Stahnke et al. (16), namely that HL glycosylation per se is not essential for the expression of catalytic activity. Evidently, the small amount of secreted rHLm2 is able to assume a proper conformation, resulting in its ability to hydrolyze the triglyceride substrate. Nevertheless, our findings clearly indicate that the majority of rHLm2 is inactive and retained intracellularly, similar to unglycosylated LPL and human HL.

Concluding remarks

We have attempted in this study to assess systematically the importance of glycosylation in the formation of a functional lipase. Our data indicate that, in general, both LPL and HL share similar requirements for Asn-linked glycosylation. In particular, lack of glycosylation at the conserved site of the N-terminal domain of both lipases disrupts, albeit to different degrees, catalytic activity and secretion. This does not imply that the glycan chains at this conserved site play a specific role in substrate catalysis; rather, we have interpreted these results as indicating the nature of glycosylation as a necessary determinant of proper protein folding. As discussed above, the addition of core oligosaccharide chains to nascent glycoproteins is often a crucial step in the formation of a correct three-dimensional structure. This appears to be the case for the conserved site at the N-terminal domain of both LPL and HL. HL does appear to have a greater flexibility than LPL, since a small number of molecules can assume a proper conformation despite the absence of glycosylation at this important site. Nonetheless, our results clearly point to the importance of glycosylation for the majority of HL molecules. We view this fact as reflecting the similarity, and not differences, between these two members of the lipase gene family.

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REFERENCES

1. Garfinkel, A. S., and M. C. Schotz. 1987. Lipoprotein Lipase. *In* Plasma Lipoproteins. A. M. Gotto, Jr., editor. Elsevier Science Publishers B. V., Amsterdam, New York, Oxford. 335–357.
2. Eckel, R. H. 1989. Lipoprotein lipase: a multifunctional enzyme relevant to common metabolic diseases. *N. Engl. J. Med.* **320:** 1060–1068.
3. Bensadoun, A. 1991. Lipoprotein lipase. *Annu. Rev. Nutr.* **11:** 217–237.
4. Jackson, R. L. 1983. Lipoprotein lipase and hepatic lipase. *In* The Enzymes, Vol. XVI. P. D. Boyer, editor. Academic Press, Inc., New York. 141–180.
5. Doolittle, M. H., H. Wong, R. C. Davis, and M. C. Schotz. 1987. Synthesis of hepatic lipase in liver and extrahepatic tissues. *J. Lipid Res.* **28:** 1326–1333.
6. Persoon, N. L. M., W. C. Hülsmann, and H. Jansen. 1986. Localization of the salt-resistant heparin-releasable lipase in the rat liver, adrenal and ovary. *J. Cell Biol.* **41:** 134–137.
7. Kirchgessner, T. G., J-C. Chuat, C. Heinzmann, J. Etienne, S. Guilhot, K. Svenson, D. Ameis, C. Pilon, L. D'Auriol, A. Andalibi, M. C. Schotz, F. Galibert, and A. J. Lusis. 1989. Organization of the human lipoprotein lipase gene and evolution of the lipase gene family. *Proc. Natl. Acad. Sci. USA.* **86:** 9647–9651.
8. Hide, W. A., L. Chan, and W-H. Li. 1992. Structure and evolution of the lipase superfamily. *J. Lipid Res.* **33:** 167–177.
9. Masuno, H., C. J. Schultz, J. W. Park, E. J. Blanchette-Mackie, C. Mateo, and R. O. Scow. 1991. Glycosylation, activity and secretion of lipoprotein lipase in cultured brown adipocytes of newborn mice. *Biochem. J.* **277:** 801–809.
10. Olivecrona, T., S. S. Chernick, G. Bengtsson-Olivecrona, M. M. Garrison, and R. O. Scow. 1987. Synthesis and secretion of lipoprotein lipase in 3T3-L1 adipocytes. *J. Biol. Chem.* **262:** 10748–10759.
11. Friedman, G., T. Chajek-Shaul, R. Gallily, O. Stein, E. Shiloni, J. Etienne, and Y. Stein. 1988. Modulation of lipoprotein lipase activity in mouse peritoneal macrophages by recombinant human tumor necrosis factor. *Biochim. Biophys. Acta.* **963:** 201–207.
12. Masuno, H., E. J. Blanchette-Mackie, C. J. Schultz, A. E. Spaeth, R. O. Scow, and H. Okuda. 1992. Retention of glucose by N-linked oligosaccharide chains impedes expression of lipoprotein lipase activity: effect of castanospermine. *J. Lipid Res.* **33:** 1343–1349.
13. Leitersdorf, E., O. Stein, and Y. Stein. 1984. Synthesis and secretion of triacylglycerol lipase by cultured rat hepatocytes. *Biochim. Biophys. Acta.* **794:** 261–268.
14. Ong, J. M., and P. A. Kern. 1989. The role of glucose and glycosylation in the regulation of lipoprotein lipase synthesis and secretion in rat adipocytes. *J. Biol. Chem.* **264:** 3177–3182.
15. Semenkovich, C. F., C-C. Luo, M. K. Nakanishi, S-H. Chen, L. C. Smith, and L. Chan. 1990. In vitro expression and site-specific mutagenesis of the cloned human lipoprotein lipase gene. *J. Biol. Chem.* **265:** 5429–5433.
16. Stahnke, G., R. C. Davis, M. H. Doolittle, H. Wong, M. C. Schotz, and H. Will. 1991. Effect of N-linked glycosylation on hepatic lipase activity. *J. Lipid Res.* **32:** 477–484.
17. Wölle, J., H. Jansen, L. C. Smith, and L. Chan. 1993. Functional role of N-linked glycosylation in human hepatic lipase: asparagine-56 is important for both enzyme activity and secretion. *J. Lipid Res.* **34:** 2169–2176.
18. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* **74:** 5463–5467.
19. Wion, K. L., T. G. Kirchgessner, A. J. Lusis, M. C. Schotz,

and R. M. Lawn. 1987. Human lipoprotein lipase complementary DNA sequence. *Science.* **235:** 1638–1641.
20. Komaromy, M. C., and M. C. Schotz. 1987. Cloning of rat hepatic lipase cDNA: evidence for a lipase gene family. *Proc. Natl. Acad. Sci. USA.* **84:** 1526–1530.
21. Taylor, J. W., W. Schmidt, R. Cosstick, A. Okruszek, and F. Eckstein. 1985. The use of phosphorothioate-modified DNA in restriction enzyme reactions to prepare nicked DNA. *Nucleic Acids Res.* **13:** 8749–8764.
22. Taylor, J. W., J. Ott, and F. Eckstein. 1985. The rapid generation of oligonucleotide-directed mutations using phosphorothioate-modified DNA. *Nucleic Acids Res.* **13:** 8764–8785.
23. Nakamaye, K., and F. Eckstein. 1986. Inhibition of restriction endonuclease Nci I cleavage by phosphorothioate groups and its application to oligonucleotide-directed mutagenesis. *Nucleic Acids Res.* **14:** 9679–9698.
24. Sprague, J., J. H. Condra, H. Arnheiter, and R. A. Lazzarini. 1983. Expression of a recombinant DNA gene coding for the vesicular stomatitis virus nucleocapsid protein. *J. Virol.* **45:** 773–781.
25. Stahnke, G., R. Sprengel, J. Augustin, and H. Will. 1987. Human hepatic triglyceride lipase: cDNA cloning, amino acid sequence and expression in a cultured cell line. *Differentiation.* **35:** 45–52.
26. Martin, G. A., S. J. Busch, G. D. Meredith, A. D. Cardin, D. T. Blankenship, S. J. T. Mao, A. E. Rechtin, C. W. Woods, M. M. Racke, M. P. Schafer, M. C. Fitzgerald, D. M. Burke, M. A. Flanagan, and R. L. Jackson. 1988. Isolation and cDNA sequence of human postheparin plasma hepatic triglyceride lipase. *J. Biol. Chem.* **263:** 10907–10914.
27. Stanssens, P., C. Opsomer, Y. M. McKeown, W. Kramer, M. Zabeau, and H-J. Fritz. 1989. Efficient oligonucleotide-directed construction of mutations in expression vectors by the gapped duplex DNA method using alternating selectable markers. *Nucleic Acids Res.* **17:** 4441–4454.
28. Cormack, B. 1991. Introduction of a point mutation by sequential PCR steps. *In* Current Protocols in Molecular Biology. F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl, editors. John Wiley and Sons, New York. 8.5.7–8.5.8.
29. Nilsson-Ehle, P., and M. C. Schotz. 1976. A stable, radioactive substrate emulsion for assay of lipoprotein lipase. *J. Lipid Res.* **17:** 536–541.
30. Goers, J. W., M. E. Pedersen, P. A. Kern, J. Ong, and M. C. Schotz. 1987. An enzyme-linked immunoassay for lipoprotein lipase. *Anal. Biochem.* **166:** 27–35.
31. Loeken, M. R., and T. F. Roth. 1983. Analysis of maternal IgG subpopulations which are transported into the chicken oocyte. *Immunology.* **49:** 21–27.
32. Strebel, K., E. Beck, K. Strohmaier, and H. Schaller. 1986. Characterization of foot-and-mouth disease virus gene products with antisera against bacterially synthesized fusion proteins. *J. Virol.* **57:** 983–991.
33. Ben-Zeev, O., M. H. Doolittle, R. C. Davis, J. Elovson, and M. C. Schotz. 1992. Maturation of lipoprotein lipase: expression of full catalytic activity requires glucose trimming but not translocation to the *cis*-Golgi compartment. *J. Biol. Chem.* **267:** 6219–6227.
34. Schneider, C., R. A. Newman, D. R. Sutherland, U. Asser, and M. F. Greaves. 1982. A one-step purification of membrane proteins using a high efficiency immunomatrix. *J. Biol. Chem.* **257:** 10766–10769.
35. Harlow, E., and D. Lane. 1988. Immunoassay. *In* Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory, New York. 553–612.
36. Vannier, C., and G. Ailhaud. 1989. Biosynthesis of lipoprotein lipase in cultured mouse adipocytes. II. Processing, subunit assembly, and intracellular transport. *J. Biol. Chem.* **264:** 13206–13216.
37. Datta, S., C-C. Luo, W-H. Li, P. Van Tuinen, D. H. Ledbetter, M. A. Brown, S-H. Chen, S-W. Liu, and L. Chan. 1988. Human hepatic lipase: cloned cDNA sequence, restriction fragment length polymorphisms, chromosomal localization, and evolutionary relationships with lipoprotein lipase and pancreatic lipase. *J. Biol. Chem.* **263:** 1107–1110.
38. De Caro, J. D., M. Boudouard, J. Bonicel, A. A. Guidoni, P. A. Desnuelle, and M. Rovery. 1981. Porcine pancreatic lipase. Completion of the primary structure. *Biochim. Biophys. Acta.* **671:** 129–138.
39. Mickel, F. S., F. Weidenbach, B. Swarovsky, K. S. LaForge, and G. A. Scheele. 1989. Structure of the canine pancreatic lipase gene. *J. Biol. Chem.* **264:** 12895–12901.
40. Lombardo, D., C. Chapus, Y. Bourne, and C. Cambillau. 1989. Crystallization and preliminary X-ray study of horse pancreatic lipase. *J. Mol. Biol.* **205:** 259–261.
41. Derewenda, Z. S., and C. Cambillau. 1991. Effects of gene mutations in lipoprotein and hepatic lipases as interpreted by a molecular model of the pancreatic triglyceride lipase. *J. Biol. Chem.* **266:** 23112–23119.
42. Lalouel, J-M., D. E. Wilson, and P-H. Iverius. 1992. Lipoprotein lipase and hepatic triglyceride lipase: molecular and genetic aspects. *Curr. Opin. Lipidol.* **3:** 86–95.
43. Winkler, F. K., A. D'Arcy, and W. Hunziker. 1990. Structure of human pancreatic lipase. *Nature.* **343:** 771–774.
44. Wong, H., R. C. Davis, J. Nikazy, K. E. Seebart, and M. C. Schotz. 1991. Domain exchange: characterization of a chimeric lipase of hepatic lipase and lipoprotein lipase. *Proc. Natl. Acad. Sci. USA.* **88:** 11290–11294.
45. Faustinella, F., L. C. Smith, C. F. Semenkovich, and L. Chan. 1991. Structural and functional roles of highly conserved serines in human lipoprotein lipase: evidence that serine 132 is essential for enzyme catalysis. *J. Biol. Chem.* **266:** 9481–9485.
46. Davis, R. C., H. Wong, J. Nikazy, K. Wang, Q. Han, and M. C. Schotz. 1992. Chimeras of hepatic lipase and lipoprotein lipase: domain localization of enzyme-specific properties. *J. Biol. Chem.* **267:** 21499–21504.
47. Lowe, M. E. 1992. The catalytic site residues and interfacial binding of human pancreatic lipase. *J. Biol. Chem.* **267:** 17069–17073.
48. Paulson, J. C. 1989. Glycoproteins: what are the sugar chains for? *Trends Biochem. Sci.* **14:** 272–276.
49. Helenius, A., T. Marquardt, and I. Braakman. 1992. The endoplasmic reticulum as a protein-folding compartment. *Trends Cell Biol.* **2:** 227–231.
50. Gething, M-J., and J. Sambrook. 1992. Protein folding in the cell. *Nature.* **355:** 33–45.
51. Rothman, J. E. 1987. Protein sorting by selective retention in the endoplasmic reticulum and Golgi stack. *Cell.* **50:** 521–522.
52. Hubbard, S. C. 1988. Regulation of glycosylation: the influence of protein structure on N-linked oligosaccharide processing. *J. Biol. Chem.* **263:** 19303–19317.
53. Marquardt, T., and A. Helenius. 1992. Misfolding and aggregation of newly synthesized proteins in the endoplasmic reticulum. *J. Cell Biol.* **117:** 505–513.
54. Hammond, C., I. Braakman, and A. Helenius. 1994. Role of N-linked oligosaccharide recognition, glucose trimming, and calnexin in glycoprotein folding and quality control. *Proc. Natl. Acad. Sci. USA.* **91:** 913–917.