# Effect of N-linked glycosylation on hepatic lipase activity

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**Abstract** Hepatic lipase (HL) is a secretory protein synthesized in hepatocytes and bound to liver endothelium. Previous studies have suggested that HL N-linked glycans are required for catalytic activity. To directly test this hypothesis, *Xenopus laevis* oocytes were used to express native rat HL or HL lacking one or both N-linked glycosylation sites. The expressed and secreted native HL had an apparent molecular mass of 53 kDa, consistent with purified rat liver HL. The mutant lacking both glycosylation sites, while poorly secreted, had an apparent molecular mass of 48 kDa, the same size observed for HL after enzymatic removal of N-linked oligosaccharides. Mutants lacking one of the two sites were intermediate in size and showed reduced secretion. Each of these expressed and secreted proteins had full catalytic activity that was inhibited by antisera to rat HL. Thus, N-linked glycosylation of rat HL, while important to lipase secretion, is not essential for the expression of lipase activity.—**Stahnke, G., R. C. Davis, M. H. Doolittle, H. Wong, M. C. Schotz, and H. Will.** Effect of N-linked glycosylation on hepatic lipase activity. *J. Lipid Res.* 1991. **32:** 477–484.

**Supplementary key words** Oligonucleotide-directed mutagenesis • *Xenopus* oocyte expression • processing and secretion

Hepatic lipase (HL) catalyzes the hydrolysis of mono-, di-, and triglycerides as well as phospholipids of circulating lipoproteins, particularly the very low density and high density lipoproteins (1). DNA sequence homologies have shown that HL is a member of a gene family that includes lipoprotein lipase (LPL) (2) and pancreatic lipase (3–6). However, in contrast to these other lipases, the precise in vivo function of HL in lipoprotein metabolism is unclear. To understand better the function of HL at the molecular level, we have recently isolated and expressed both the rat (7, 8) and human (3) HL cDNAs (Stahnke, G., E. von Hodenberg, and H. Will, unpublished data).

Rat HL (rHL) contains two potential N-linked glycosylation sites (7, 9), at Asn 57 and Asn 376, both of which are used (10). These two sites in rat HL are conserved in the human enzyme (Asn 55 and Asn 374); two additional N-linked glycosylation sites in human HL are present at Asn 19 and Asn 339 (3, 11, 12). Indirect evidence suggests that N-linked glycosylation of rHL is an essential posttranslational step for attainment of enzyme activity. In particular, a study by Leitersdorf, Stein, and Stein (13) reported that rHL activity of hepatocytes was markedly reduced after incubation with tunicamycin, an inhibitor of N-linked glycosylation. Activity of the structurally related LPL also seems to depend on N-linked glycans. For instance, Ong and Kern (14) found that cultured rat adipocytes deprived of glucose synthesized a non-glycosylated form of LPL that was not secreted; the non-glycosylated enzyme, retained intracellularly, had no lipolytic activity. More recently, human LPL expressed transiently in COS cells was shown to be inhibited in both activity and secretion when one of the N-linked glycosylation sites (Asn 43) was inactivated by site-directed mutagenesis (O. Ben-Zeev, unpublished data and (15)). In contrast to HL and LPL, the remaining member of the lipase gene family (pancreatic lipase) does not appear to require glycan groups for activity, since in several species (horse, cow, and sheep), pancreatic lipase (16, 17) is not glycosylated.

Our goal was to determine directly the effect of glycosylation on rat HL activity. The approach used was to alter potential glycosylation sites by oligonucleotide-directed mutagenesis, and to express native HL and HL lacking one or both glycosylation sites in *Xenopus* oocytes.

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Abbreviations: HL, hepatic lipase; rHL, rat hepatic lipase; LPL, lipoprotein lipase; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; RIA, radioimmunoassay; TCA, trichloroacetic acid; PMSF, phenylmethylsulfonylfluoride; SE, standard error; MBS, modified Barths' saline.

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## MATERIALS AND METHODS

### Construction of mutated rat HL clones

The full length rat HL cDNA clone (7) was transferred to the EcoRI site of Phagescript (Strategene), a single-stranded M13-based vector. Oligonucleotide-directed mutagenesis (18–20) was performed using materials and instructions from Amersham. After verification of the mutant sequences by dideoxy chain termination (21), the mutant cDNAs were transferred to the plasmid vector pSP64 (Pharmacia). All mutants were again sequenced in the plasmid vector to verify the presence of the desired mutation.

### In vitro synthesis of rHL mRNA

After purification by cesium chloride-ethidium bromide equilibrium centrifugation, HL pSP64 plasmid constructs were linearized at the unique BamHI site, leaving 19 bp of linker sequence attached to the 3' terminus of the cDNA insert. In vitro transcription was performed with SP6 RNA polymerase (Biolabs, New England) (22). Briefly, 0.5–1 μg pSP64 DNA template was incubated for 1 h at 40°C in 40 mM Tris, pH 7.5, containing 6 mM $MgCl_2$, 10 mM dithiothreitol, 4 mM spermidine, 0.5–1 μg HL pSP64 DNA template, 0.5 mM each of ATP, CTP, GTP, and UTP, 30 units RNasin, 5 units SP6 RNA polymerase, and 0.14 mM 7mGpppG in a total volume of 40 μl. The DNA template was removed by digestion with 3 units RNase-free DNase 1 (Boehringer Mannheim) for 7 min at 37°C and RNA transcripts were purified by phenol–chloroform extraction and ethanol precipitation. RNA size homogeneity was verified on a vertical 1% agarose gel.

### *Xenopus laevis* oocytes

Oocytes were isolated (23) and large oocytes were selected, washed twice, and kept overnight at 20°C in modified Barths' saline (MBS). MBS contains 15 mM HEPES-NaOH or Tris-HCl (pH 7.6), containing 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.30 mM $CaNO_3$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 10 μg/ml sodium penicillin, 10 μg/ml streptomycin sulfate, 100 μg/ml gentamycin, and 20 units/ml nystatin.

Oocytes were injected with either 25 nl water or 25 nl water containing 25 ng RNA transcript using a microinjector with a glass capillary 10–20 μm in diameter. Injected oocytes were kept overnight in MBS at 20°C. The oocytes were washed three times with MBS, and distributed in 96-well culture dishes (Costar) previously treated with bovine serum albumin (0.5 mg/ml). Each well contained 5 oocytes and was incubated in 30 μl incubation medium (MBS, containing 10 mg/ml bovine serum albumin, 50 units/ml heparin (Liquemin, Roche), 0.22 units/ml aprotinin (Sigma), and 1 μCi/μl [$^{35}$S]methionine or 0.5 μCi/μl [$^{3}$H]leucine (Amersham Buchler)). After 24 h at 20°C, media from four wells were pooled and stored at −70°C until analyzed. Oocytes of corresponding wells were also pooled and washed once in phosphate-buffered saline prior to freezing. Oocytes were homogenized by resuspending 20 oocytes in 300 μl of 5 mM sodium barbital, pH 8.3, containing 0.15 M NaCl and 1 mg/ml heparin. Samples were sonicated for 0.5 sec at 1 *g* force at 4°C. Aliquots were removed for activity assay and immunoprecipitation.

### Immunoprecipitation

Triton X-100 was added to the oocyte sonicates to a final concentration of 3% (v/v). Nuclei and debris were removed by centrifugation at 10,000 *g* for 10 min at 4°C. Fifty μl of the supernatant was added to 450 μl of lysis buffer (3% Triton X-100, 0.1% lauroyl sarcosine, 1 mM PMSF, 10 μg/ml cyclohexamide in 0.1 M Tris, pH 7.5). A two-cycle immunoprecipitation of HL was performed (8) using 10 μl of rabbit anti-rat HL antiserum (10). Further immunoprecipitation of the medium precipitated no additional HL.

SDS-PAGE and fluorography were performed as described (10). Dried gels were exposed to Kodak X-Omat AR film overnight ($^{35}$S experiments) or 3–4 days ($^{3}$H experiments) with intensifying screens at −80°C. Based on the mobility of immunoprecipitated HL observed in the fluorogram, appropriate gel regions were excised and incubated overnight in the presence of 1 ml of 80% Protosol (DuPont-New England Nuclear). Radioactivity was measured by liquid scintillation spectrometry. Incorporation of radiolabeled amino acid into total protein was determined by precipitation of total protein by trichloroacetic acid (24).

### Lipase assay procedure

HL activity was assayed using as substrate a triolein-lysolecithin emulsion (10). The assay was modified as follows: instead of determining the radioactivity present in 1 ml of the aqueous layer, 2 ml were removed and acidified with 200 μl concentrated HCl, followed by extraction with 2 ml hexane. The samples were vigorously mixed and centrifuged at 1500 *g* for 10 min. The hexane phase (1 ml) was removed, dried under $N_2$, and resuspended in 30 μl $CHCl_3$. Samples and lipid standards were chromatographed on thin-layer silica plates (Whatman, LK6D) with hexane–ethyl ether–acetic acid 3:1:0.1; standards were visualized by $I_2$ vapors. Regions corresponding to free fatty acid were scraped from the plate and radioactivity was determined by liquid scintillation spectrometry.

## RESULTS

N-linked oligosaccharides are attached via a glycosylamine linkage to the amide nitrogen of an asparagine residue within the consensus sequence Asn-Xaa-Ser(Thr), where Xaa is any amino acid but proline (25, 26). The two such asparagine residues in rHL, Asn 57 and Asn 376, were replaced by altering the Asn codon to one specifying glutamine. The resulting mutant constructs, 57NQ, 376NQ, and 57+376NQ (**Fig. 1**), were expressed in *Xenopus* oocytes.

Oocytes expressing native rHL secreted a single immunoprecipitable protein of 53 kDa (**Fig. 2**), consistent with the molecular weight of purified rat liver HL (27). Furthermore, incubation of rHL antiserum with purified rHL competed with the immunoprecipitation of the oocyte-expressed protein. In contrast, oocytes injected with water did not synthesize an immunoprecipitable protein comigrating with rHL. Rat HL within the oocytes showed two molecular mass forms, one 53 kDa and a second 51 kDa. This is consistent with our previous observation of HL synthesis in rat hepatocytes (10) where fully processed HL is 53 kDa and the high mannose form migrates at 51 kDa. The presence of two intracellular forms of rHL and the fully processed secreted form is also consistent with findings of other investigators examining rHL expression in liver, hepatocytes, and hepatoma cells (9). Thus, oocyte-expressed proteins produced from rHL RNA transcripts appear to mimic, in size and form, the enzyme produced in vivo.

Oocytes expressing mutants 57NQ, 376NQ, and 57+376NQ secreted immunoprecipitable proteins with reduced size consistent with the sequential loss of N-linked oligosaccharides (**Fig. 3**). The mutant protein lacking both glycan chains, 57+376NQ, had an apparent molecular mass of 48 kDa, identical in size to the normal rat enzyme following removal of all N-linked oligosaccharides (10). The two other mutant proteins, 57NQ and 376NQ, were intermediate in size between 57+376NQ and native rHL. These data indicate that the injected oocytes express native rHL as the fully glycosylated enzyme and that replacement of either Asn 57 or Asn 376 with glutamine prevents N-linked glycosylation at these positions.

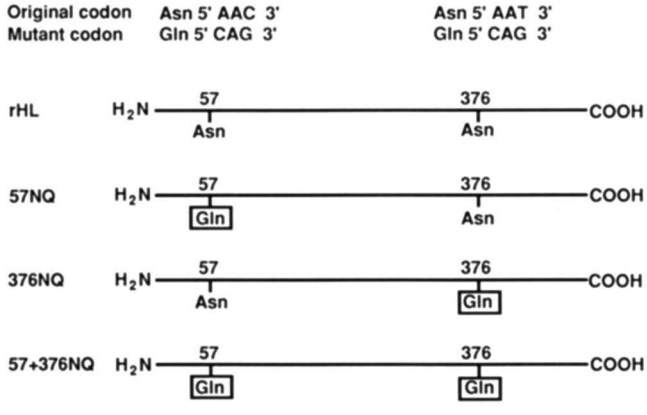


**Fig. 1.** Site-specific mutations of rHL glycosylation sites. Glycosylated Asn residues at amino acid positions 57 and 376 in rHL are indicated. Presented at the top are the native 57 and 376 Asn codons and their corresponding Gln codons following site-directed mutagenesis. Shown below are the constructs of the single mutants, 57NQ and 376NQ, and the double mutant, 57+376NQ.

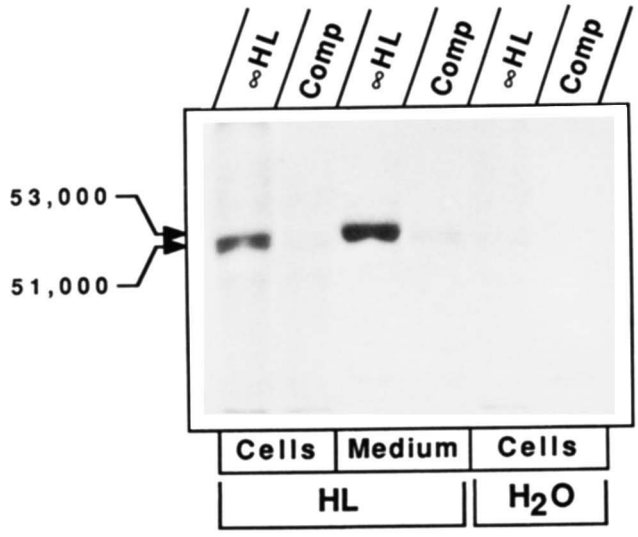


**Fig. 2.** Expression of rHL in *Xenopus* oocytes. Shown is a fluorogram of an SDS polyacrylamide gel of HL immunoprecipitated from both [$^{35}$S]methionine-labeled oocyte homogenates (cells) and medium after injection of native rHL transcripts (HL) or water ($H_2O$); αHL, immunoprecipitation after incubation with rabbit anti-rat HL antiserum; Comp, competition assay performed by incubating HL antiserum with purified, unlabeled rHL prior to immunoprecipitation. The molecular weights of the complex and high mannose forms of HL (53,000 and 51,000, respectively) are indicated.

To assess whether N-linked glycosylation is required for expression of rHL catalytic activity, the mutant proteins 57NQ, 376NQ, and 57+376NQ were assayed for the hydrolysis of an emulsified triolein substrate (**Fig. 4**). After injection of oocytes with transcripts for rHL and in the presence of heparin, the level of lipase activity secreted to the medium was similar to that retained in the oocyte, about 1700 pmol free fatty acid hydrolyzed per h per oocyte (Fig. 4). While all the mutants hydrolyzed substrate, the activity levels were lower than that of native rHL, particularly for the mutant lacking both N-linked glycans (57+376NQ). For the 57NQ and 376NQ mutants, activity in the medium was about half that found inside the cells, whereas the medium activity of the 57+376NQ mutant was less than 25% of that within the oocyte (Fig. 4). Although there was a quantitative decrease, the activity measured in the medium was due to HL, as judged by antibody inhibition (**Fig. 5**). The medium of oocytes expressing native rHL and glycosyla-

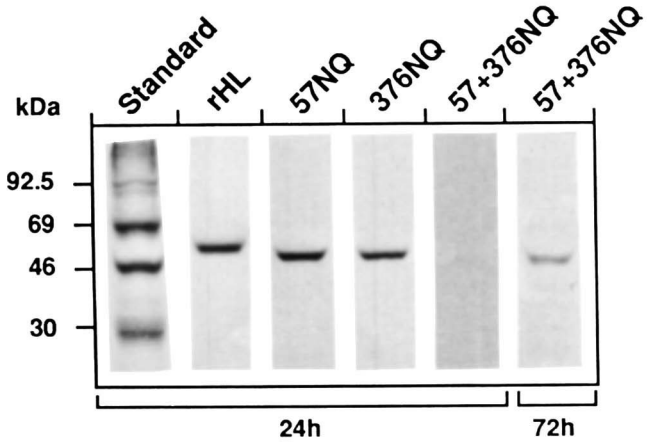


**Fig. 3.** Expression of native rHL and glycosylation-deficient mutants. Incubation medium of [$^{35}$S]methionine-labeled oocytes injected with native and mutant constructs (see Fig. 1) was immunoprecipitated. Shown are the fluorograms of SDS polyacrylamide gels following exposure to Kodak XAR film for 24 or 72 h. The molecular weights of the native and mutant proteins are: 53,000 for rHL; 51,000 for 57NQ and 376NQ; and 48,000 for 57+376NQ. The migration positions of $\beta$-galactosidase (92.5 kDa), bovine serum albumin (69 kDa), ovalbumin (46 kDa), and carbonic anhydrase (30 kDa) are indicated.

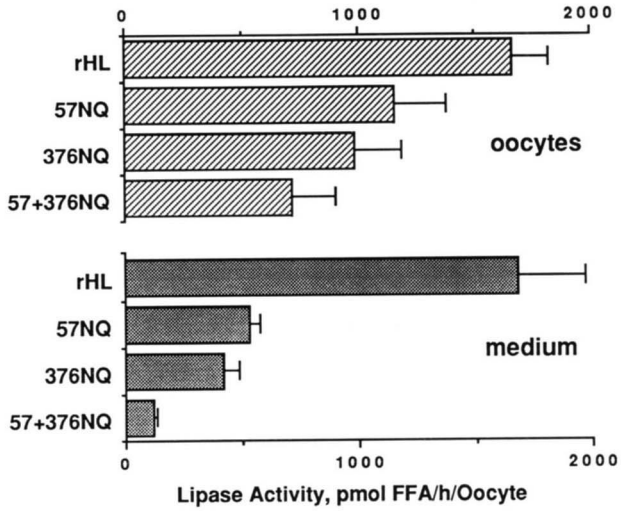


**Fig. 4.** Comparison of the lipolytic activity of native rHL and glycosylation-deficient mutants. Oocytes and medium from pools of 20 oocytes injected with rHL, 57NQ, 376NQ, and 57+376NQ transcripts were assayed for triolein hydrolysis as described under Materials and Methods. The bars indicate the mean ± SE of lipase activity, measured as pmol free fatty acid released per h per oocyte after subtraction of background; background levels of lipolysis found in the cell medium and homogenates of water-injected samples were less than 10% of these values.

tion mutants 57NQ, 376NQ, or 57+376NQ were assayed following incubation with either immune or pre-immune serum; all oocyte-expressed lipase activities were specifically suppressed by over 90% by rHL antiserum (Fig. 5). Intracellular lipase activity for each mutant was also inhibited by rHL antiserum (data not shown).

The decreased activity levels in the medium of oocytes expressing the glycosylation mutants could be due to at least two factors: *i*) the mutant forms are not secreted as efficiently as the native enzyme, resulting in lower levels of active lipase; or *ii*) lipase of lower specific activity is secreted due to the essential requirement of glycosylation for attainment of full catalytic activity. These possibilities were examined by estimating lipase protein mass. Judging from the levels of expressed activity and using the known specific activity for rHL (27), the maximum concentration of oocyte-expressed lipase was calculated to be about 2 ng/ml. This value is below the sensitivity of available ELISA and RIA; therefore, in a separate experiment using [$^{35}$S]methionine, rHL protein levels were estimated by incorporation of radiolabeled amino acid into immunoprecipitable rHL after a 24-h pulse-labeling. Full precipitation of each protein was ensured by using saturating levels of antibody. As shown in **Table 1,** incorporation rates of radiolabeled methionine into total acid-precipitable oocyte proteins were similar between oocytes injected with water, rHL, 57NQ, 376NQ, or 57+376NQ transcripts. This indicates that the radiospecific activity of the intracellular methionine pool used for synthesis of oocyte-encoded proteins, as well as injected transcripts, remained constant. Thus, incorporation of radiolabeled methionine into immunoprecipitable HL was proportional to HL mass synthesized during the labeling period.

**Fig. 6** shows expressed rHL protein in oocyte homogenates and incubation medium after labeling for 24 h. Similar to the expression pattern for lipolytic activity (Fig. 4), lipase mass was highest in the medium of rHL, was intermediate in the single mutants and was decreased by over 95% in the medium of the 57+376NQ mutant. This suggests that the low activity level in the

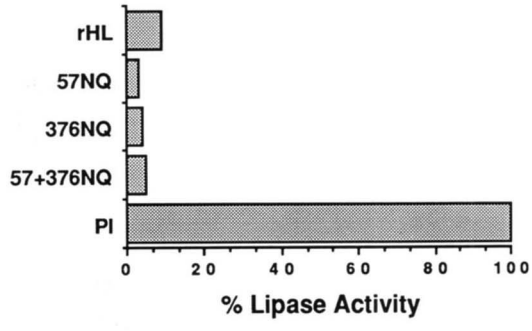


**Fig. 5.** Immunoinhibition of lipolytic activity. The media of oocytes expressing native rHL and glycosylation-deficient mutants (Fig. 1) were incubated with either preimmune serum or rabbit anti-rat HL antiserum prior to assaying for triolein hydrolysis as described under Materials and Methods. The bars represent the percent lipolytic activity remaining after the incubation with antiserum. PI, the lipase activity in the presence of preimmune serum, is defined as 100%. Actual values of lipase activity (nmol free fatty acid released per h per oocyte) were: 3.16 for rHL; 2.75 for 57NQ, 2.68 for 376NQ; and 0.55 for 57+376NQ.

TABLE 1. Relative specific activity of native rHL and glycosylation deficient mutants

| Sample | Lipase Activity | Relative HL Mass | Incorporation Total Protein | Relative Specific Activity |
|---|---|---|---|---|
| | *pmol FFA/h/oocyte* | *cpm/oocyte* | *cpm/oocyte × 10⁻⁶* | |
| rHL (n = 3) | 5120 ± 221 | 3330 ± 478 | 5.7 ± 0.65 | 100 ± 14 |
| 57NQ (n = 4) | 2065 ± 369 | 530 ± 33 | 6.3 ± 0.35 | 274 ± 47 |
| 376NQ (n = 3) | 2103 ± 657 | 1310 ± 150 | 6.2 ± 0.12 | 106 ± 25 |
| 57 + 376NQ (n = 3) | 170 ± 12 | 100 ± 8 | 5.8 ± 0.74 | 110 ± 14 |

Lipolytic activity, relative HL mass, and incorporation of label into total protein were determined on identical samples from pooled media of 20 oocytes incubated for 24 h with [$^{35}$S]methionine. The multiple values given for rHL, 57NQ, 376NQ, and 57 + 376NQ represent data obtained from separate pools of 20 oocytes each, injected with water and RNA transcripts for rHL or mutant (Fig. 1). For lipase activity and incorporation of radiolabeled amino acid into total protein, each value is the average of three assays; for HL relative mass, each value is one determination of the incorporation of [$^{35}$S]methionine into immunoprecipitable HL (see Fig. 6 and Results). Measurements of water-injected oocytes were subtracted from each value; values for water-injected oocytes were less than 10% of those of other samples. Relative specific activity was calculated by dividing lipase activity by relative HL mass, the latter value represented as a percentage of total protein synthesis (i.e., corrected for changes in the incorporation of [$^{35}$S]methionine into total protein). Relative specific activities are normalized to native rHL, represented as 100. All data are shown as average ± SE.

medium of the 57+376NQ mutant shown in Fig. 4 is most likely due to the impairment of its secretion, and does not result from the presence of inactive mass.

Compared to the medium, HL activity inside the oocytes (Fig. 4) is proportionately lower than the respective HL protein mass, as measured by immunoprecipitation (Fig. 6). This discrepancy most likely results from differences in the preparation of oocytes used for activity assays versus immunoprecipitation. Since HL activity assay is extremely sensitive to the presence of detergents, HL activity was determined from sonicates of oocytes prepared in detergent-free buffer, whereas Triton X-100 was added to sonicates used for immunoprecipitation. It is likely that oocytes disrupted in the absence of detergent did not release total intracellular HL, particularly that fraction sequestered within membrane-bound vesicles. Thus, while the presence of latent lipase activity would not be detected in the activity assay, the addition of detergent to sonicates would have made latent lipase available for immunoprecipitation.

Since the conditions for the measurement of enzyme activity and immunoprecipitation were identical for secreted HL in the medium, the "relative" enzyme specific activity of rHL, 57NQ, 376NQ, and 57+376NQ was calculated by dividing lipase catalytic activity by the level of radiolabeled amino acid incorporated into HL, as a proportional estimate of lipase mass (Table 1). Radiolabeled amino acid incorporation into HL (normalized to that incorporated into total proteins to compensate for any variation in methionine radiospecific activity) was used as an estimate of HL mass. The calculated "relative" specific activity (Table 1) is essentially the same for rHL, 376NQ, and 57+376NQ, whereas it is increased by almost threefold for mutant 57NQ. The increase in specific activity for 57NQ may reflect better utilization of the artificial substrate used in the assay. From this estimate of enzyme specific activity, it is clear that HL expressed as a fully glycosylated, partially glycosylated, or non-glycosylated protein is fully active. Thus, while glycosylation appears to play an important role in HL secretion, it is not essential for the expression of catalytic activity.

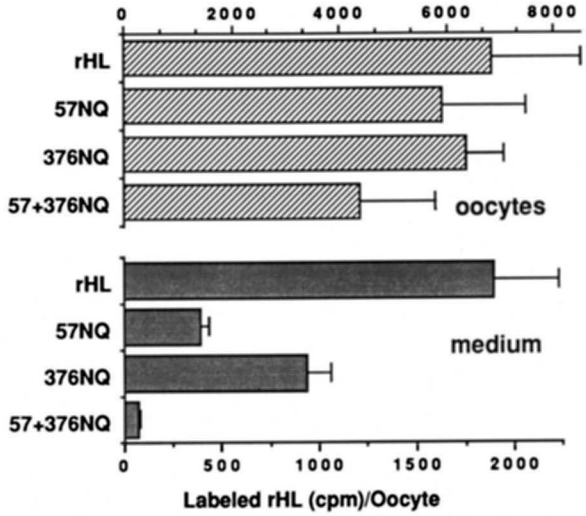


**Fig. 6.** Comparison of the relative HL protein levels of native rHL and glycosylation-deficient mutants. Relative HL protein levels were estimated by the incorporation of [$^{35}$S]methionine into rHL and mutant proteins following a 24 h period of pulse-labeling (see Results). Oocyte sonicates were treated with detergent prior to immunoprecipitation. After SDS polyacrylamide gel electrophoresis and fluorography, immunoprecipitated HL bands corresponding to rHL, 57NQ, 376NQ, and 57+376NQ were excised and radioactivity was determined as described under Materials and Methods. The results given are the mean values ± SE of [$^{35}$S]methionine incorporated into HL immunoprecipitable protein per oocyte.

## DISCUSSION

HL is a member of a lipase gene family (3, 7, 11, 12, 28), whose other members are LPL (2, 29) and pancreatic lipase (4–6, 29). All three lipases share a highly conserved central region, presumably critical for lipase functions. Members of this gene family are also homologous with respect to location of cysteine residues and glycan attachment sites. This report examines the role of glycosylation in HL activity, as an initial attempt to determine the role of glycosylation in catalysis among members of this gene family.

There is much evidence suggesting that not only is glycosylation required for LPL secretion, but that it is an essential step in LPL intracellular "activation." For example, transient expression from human LPL clones that have been mutagenized to eliminate the N-linked glycosylation site at Asn 43 produces an inactive LPL protein that is not secreted (O. Ben-Zeev, unpublished data and (15)). Further, glucose or other monosaccharides that can be converted to glucose-6-phosphate must be present to support the synthesis of active LPL in rat adipose tissue (30–32), isolated rat adipocytes (14), and 3T3-L1 adipocytes (33). Tunicamycin, which blocks N-linked glycosylation, inhibits enzymatic activity but not secretion of LPL in Ob17 cells (34) and isolated rat adipocytes (14) with a concomitant loss of intracellular enzyme activity. It has been suggested that newly synthesized LPL in the endoplasmic reticulum containing oligomannosyl residues is inactive until processing of the glycan chains occurs within the Golgi (35). Like LPL, HL has also been shown to be inactive and not secreted in isolated rat hepatocytes after tunicamycin treatment (13).

Although these prior studies show that LPL and HL are inactive and retained intracellularly when glycosylation is blocked, the molecular basis of this inactivation is unclear. Tunicamycin, for example, blocks the glycosylation of all proteins, thus potentially influencing a host of regulatory and processing enzymes. The resulting inhibition of the lipases is therefore likely to be a secondary effect to general blockage of glycosylation. Inhibition of N-linked glycosylation is well known to affect the secretion of many glycoproteins (36). However, while only a small amount of the nonglycosylated 57+376NQ mutant is secreted by oocytes, we find that it is fully active. The nonessential role of glycosylation for catalytic activity in other members of this lipase gene family is further supported by the fact that equine, bovine, and ovine pancreatic lipase (16, 17) are not glycoproteins, although the putative active site, and presumably their catalytic mechanism, is conserved among the lipases of all species examined (3, 11). In addition, the partially purified high mannose form of guinea pig LPL is catalytically active (37), contrary to in vivo studies in mouse Ob 17 cells, which demonstrate an inactive LPL high mannose form (38).

The full catalytic activity of rHL glycosylation mutants is in contrast to the inactivity of the human LPL glycosylation mutant expressed in COS cells (O. Ben-Zeev, unpublished data and (15)). Preliminary experiments in COS cells suggest that this distinction does not reflect differences between the expression systems, but rather, differing properties of the rHL and human LPL enzymes (data not shown). If the highly conserved structure of the lipase gene family implies a similar conservation of functional residues in the catalytic site, then, based on our findings, the N-linked glycans should have no direct influence on catalysis in the lipase family. Since a glycosylation mutant of LPL was determined to be inactive (O. Ben-Zeev, unpublished data and (15)), then glycosylation may play an additional role in the case of LPL, such as facilitation of cofactor binding that HL does not require.

The reason for decreased expression of the glycosylation-deficient mutants is unclear. For each RNA transcript, levels of lipase synthesis were highly consistent between oocyte pools, showing that the activity differences did not arise simply from pool to pool variation in RNA levels. Although HL degradation products were not observed, it is possible that the nonglycosylated mutants are less stable. For this reason, only the full-sized immunoprecipitating protein band was used in lipase mass estimates.

In summary, HL activity and mass were found in both oocyte medium and homogenates regardless of the HL RNA injected (Fig. 4 and Fig. 6). While 50% of the total expressed activity of the native enzyme was found in the medium, 15% and 30% were located in the medium for the double (57+376NQ) and single (57NQ, 376NQ) mutants, respectively. The decreased medium levels of activity and mass of the single and double mutants relative to native enzyme most likely reflects the requirement of glycosylation for efficient secretion. Elimination of one or both HL glycan attachment sites resulted in the production of catalytically competent enzyme. Thus, the relative specific activities for nonglycosylated rHL and the native enzyme are essentially equivalent, showing unequivocally that HL oligosaccharides are not required for catalytic activity.

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