Retention of glucose by N-linked oligosaccharide chains impedes expression of lipoprotein lipase activity: effect of castanospermine

Hiroshi Masuno,[1,*] E. Joan Blanchette-Mackie,[†] Charles J. Schultz,[†] Albert E. Spaeth,[†] Robert O. Scow,[†] and Hiromichi Okuda[*]

Department of Medical Biochemistry,* School of Medicine, Ehime University, Shigenobu, Onsen-gun, Ehime 791-02, Japan, and Endocrinology Section,† Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892

Abstract The effect of castanospermine (CSTP), an inhibitor of glucosidase I, on processing, activity, and secretion of lipoprotein lipase was studied in 3T3-L1 adipocytes. Processing was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of endoglycosidase H (endo H)-digested subunits of lipoprotein lipase from cells incubated 1–2 h with [^{35}S]methionine. Lipoprotein lipase in untreated cells consisted of two groups of subunits, M_r = 55,000–58,000 and M_r = 53,000–55,000. The heavier subunits were endo H-resistant, whereas the others were either totally or partially endo H-sensitive. The lipase secreted by untreated cells contained primarily endo H-resistant subunits. Immunofluorescent studies showed that lipoprotein lipase accumulated in Golgi in untreated cells. CSTP, 100 μg/ml for 18 h, decreased intracellular lipase activity by 80% and decreased secretion of lipase activity by 91%. Most of the lipase subunits in CSTP-treated cells were totally endo H-sensitive with M_r = 57,000, some were partially endo H-sensitive, and a trace was endo-H resistant. Totally endo H-sensitive subunits in CSTP-treated cells had a M_r 2,000–4,000 larger than that in untreated cells, indicating impaired trimming of sugar residues from oligosaccharide chains of the lipase in CSTP-treated cells. The small amount of lipase secreted by CSTP-treated cells consisted primarily of partially endo H-sensitive subunits, with one sensitive and one resistant chain per subunit. Immunofluorescent studies showed that lipoprotein lipase was excluded from Golgi in CSTP-treated cells. These studies demonstrate that inhibition of glucosidase I with castanospermine blocks trimming of oligosaccharide chains of lipoprotein lipase in endoplasmic reticulum, transport of lipase from endoplasmic reticulum to Golgi, processing of oligosaccharide chains to endo H-resistance, and expression and secretion of lipoprotein lipase activity. The findings indicate that removal of the distal glucose residue from oligosaccharide chains of lipoprotein lipase is necessary for activity and secretion of the lipase.—Masuno, H., E. J. Blanchette-Mackie, C. J. Schultz, A. E. Spaeth, R. O. Scow, and H. Okuda. Retention of glucose by N-linked oligosaccharide chains impedes expression of lipoprotein lipase activity: effect of castanospermine. *J. Lipid Res.* 1992. **33:** 1343–1349.

Supplementary key words 3T3-L1 adipocytes • glucosidase I • endoglycosidase H • glycosylation • immunocytochemistry • glycoprotein transport

Lipoprotein lipase is a glycoprotein produced by parenchymal cells in extrahepatic tissues and transferred to capillaries where it hydrolyzes plasma triacylglycerol to fatty acid and monoacylglycerol (1–3). The enzyme is attached to the luminal surface of vascular endothelial cells via an interaction with membrane-bound heparan sulfate (4). Active lipoprotein lipase is a noncovalent dimer of identical glycopeptides. Dissociation of the dimeric form to the monomeric form renders the lipase inactive (4–6).

Studies in cultured adipocytes indicate that production of active lipoprotein lipase normally involves synthesis and N-linked glycosylation of lipase subunits in endoplasmic reticulum, trimming of glucose and certain mannose residues from endo H-sensitive oligosaccharide chains in endoplasmic reticulum and Golgi, processing of chains to make them endo H-resistant by addition of other sugars to the chains in Golgi, dimerization of subunits, and secretion of endo H-resistant lipase (7–12). Lipoprotein lipase in mouse adipocytes has two N-linked oligosaccharide chains per subunit (10, 11) and that in guinea pig adipocytes has three (9). Recent studies in adipocytes using inhibitors of Golgi mannosidase I (1-deoxymannojirimycin) and mannosidase II (swainsonine) showed that processing of oligosaccharide chains to endo H-resistant (complex) types is not required for activity or secretion of lipoprotein lipase (9, 12).

Brown adipocytes cultured from tissues of *cld/cld* mice synthesize lipoprotein lipase that has incompletely

Abbreviations: CSTP, castanospermine; DMEM, Dulbecco's modified Eagle's medium containing 5.5 mM glucose; PBS, Dulbecco's phosphate-buffered saline; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonyl fluoride; EDTA, ethylenediaminetetraacetic acid, disodium salt; endo H, endoglycosidase H.

[1]To whom correspondence should be addressed.

processed (high mannose-type) oligosaccharide chains, but it is inactive and is retained in endoplasmic reticulum (11). These results suggest that trimming of oligosaccharide chains in endoplasmic reticulum may be necessary for expression of lipoprotein lipase activity.

This paper describes the effect of CSTP, an inhibitor of glucosidase I in endoplasmic reticulum (13–15), on processing, activity, and secretion of lipoprotein lipase in 3T3-L1 adipocytes. Treatment of cells with CSTP resulted in synthesis of endo H-sensitive lipase which was inactive and retained in endoplasmic reticulum.

MATERIALS AND METHODS

Materials

L-[^{35}S]methionine and ENHANCE^{R} were from Du Pont-New England Nuclear. Tri[9,10(n)-^{3}H]oleoylglycerol and ^{14}C-labeled methylated proteins for molecular weight standards were from Amersham. DMEM was from Nissui Pharmaceutical Co., Tokyo. Methionine-deficient DMEM was from GIBCO. Fetal bovine serum was from M. A. Bioproducts, Walkersville, MD. Castanospermine was from Boehringer. Leupeptin and pepstatin were from Peptide Instituters, Inc., Osaka. Tunicamycin B complex, heparin, insulin, dexamethasone, antipain, benzamidine, and aprotinin were from Sigma. Endoglycosidase H was from Genzyme. Bovine serum albumin was from Wako Pure Chemicals, Co., Osaka. Rabbit anti-chicken IgG was from Pel-Freez. Chicken antiserum to bovine lipoprotein lipase was kindly given by Dr. Thomas Olivecrona, Department of Physiological Chemistry, University of Umeå, Umeå, Sweden. All other chemicals were highest quality commercially available.

Solution A, used for harvesting and suspending cells, contained 0.2 M Tris, 3% Triton X-100, 1% N-lauroylsarcosine, 0.15 M NaCl, and 1 mM PMSF at pH 7.5. Solution B, used for washing immunoprecipitates, contained 0.1 M sodium borate, 0.1% SDS, 0.5 M NaCl, 1% Triton X-100, 5 mM EDTA, 10 μg/ml leupeptin, 1 μg/ml pepstatin, 3.5 μg/ml aprotinin, and 0.1 mM PMSF at pH 8.0. Solution C, used to dissolve immunoprecipitates for SDS-PAGE, contained 0.0625 M Tris, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, and 0.001% bromophenol blue at pH 6.8.

Standard culture medium contained 10% fetal bovine serum, 100 units/ml penicillin, 0.1 mg/ml streptomycin, and 0.25 μg/ml amphotericin B in DMEM (containing 5.5 mM glucose). Complete medium contained 5 μg/ml insulin in standard culture medium.

Cell culture

3T3-L1 cells were grown to confluence with standard medium in 60-mm plates as described previously (16). Confluent cells were stimulated to differentiate into adipocytes by supplementing standard medium with 1 μM dexamethasone, 10 μg/ml insulin, and 0.5 mM methylisobutylxanthine for 2 days. The medium was then replaced with complete medium and was changed every 2 days. The cells were used for experiment on days 8–10 of confluence. Cells that were used were never passed more than ten times.

Assay of lipoprotein lipase activity

Lipoprotein lipase activity associated with cells was measured in aqueous extracts of dried-defatted (acetone/ether) powders of cells (11, 17). Lipoprotein lipase activity in the culture medium was measured in aliquots of medium filtered through 0.22-μm "Millex-GS" filter units. The assays were begun within 3 min after taking the aliquots. One milliunit of lipolytic activity represents release of 1 nmole of fatty acids/min at 37°C.

[^{35}S]methionine incorporation studies

3T3-L1 adipocytes were incubated for 16 h at 37°C in 3 ml of complete medium containing 0 or 100 μg/ml CSTP, or incubated for 21.5 h at 37°C in 3 ml of complete medium containing 1.0 μg/ml tunicamycin. Cells were then washed with PBS and incubated for 30 min at 37°C in 1.5 ml of methionine-deficient DMEM containing the appropriate additive and 2% fetal bovine serum that had been dialyzed overnight against methionine-deficient DMEM. Thirty min later, 160 μCi of [^{35}S]methionine was added to each plate and the plates were incubated for 1–2 h at 37°C. At the end of the experiment, the medium was removed and the plates were rinsed with ice-cold PBS. The cells were harvested into 0.5 ml of solution A and sonicated briefly at 0°C, the suspension was centrifuged at 15,000 *g* for 20 min at 4°C, and the infranatant was removed and stored at −80°C for immunoprecipitation studies.

The medium was filtered through 0.22-μm "Millex-GS" filter unit and proteins in the filtrate were precipitated with 10% trichloroacetic acid in the presence of 50 μg bovine serum albumin as a carrier. The precipitates were dissolved in 1 N NaOH. After neutralizing with 1 N HCl, aliquots were mixed with the same volume of double strength solution A and stored at −80°C for immunoprecipitation studies.

^{35}S-labeled lipoprotein lipase was precipitated with chicken antiserum to bovine lipoprotein lipase as described previously (11). The immunoprecipitates were washed 7 times with solution B, and once with 0.0625 M Tris-HCl buffer, pH 6.8. The washed immunoprecipitates were dissolved in solution C for SDS-PAGE in a Laemmli type system (18) with 10% acrylamide resolving gel and 3% acrylamide stacking gel. Gels were stained with Coomassie Blue, destained, impregnated with EN-

HANCE[R], and dried on a sheet of cellophane with vacuum. Autoradiographs were obtained by exposure of Kodak X-Omat film to gels at −80°C.

Enzymatic deglycosylation of lipoprotein lipase

^{35}S-labeled lipoprotein lipase immunoprecipitated with chicken antiserum to lipoprotein lipase was digested with endo H as described previously (19).

Immunolocalization of lipoprotein lipase in 3T3-L1 adipocytes

Lipoprotein lipase was detected immunocytochemically with a fluorescent double antibody technique, using chicken antiserum to bovine lipoprotein lipase and rhodamine-labeled affinity-purified rabbit antibodies to chicken IgG as described previously (11).

Chemical analysis

DNA was measured fluorometrically by the method of Hinegardner (20) using calf thymus DNA as standard.

RESULTS

Untreated 3T3-L1 adipocytes

Lipoprotein lipase activity was measured in aqueous extracts of acetone/ether powders of cells and in the culture medium. Untreated 3T3-L1 adipocytes contained 1,050 milliunits/mg DNA of lipoprotein lipase activity and spontaneously released 150 milliunits/mg DNA of activity into the medium in 2 h (**Table 1**).

Untreated 3T3-L1 adipocytes incorporated [^{35}S]methionine into total protein at a linear rate for 2 h (data not shown), indicating that the amount of radioactivity added to the medium was sufficient to label proteins synthesized by the cells.

TABLE 1. Effect of CSTP on lipoprotein lipase activity in 3T3-L1 adipocytes

Concentration of CSTP in Medium	Lipoprotein Lipase Activity	
	In Cells	In Medium at 2 h
μg/ml	*milliunits/mg DNA*	
0	1048 ± 58	152 ± 16
5	944 ± 104	152 ± 18
10	843 ± 57	114 ± 13
100	205 ± 22	14 ± 1

The culture medium was removed from plates of 3T3-L1 adipocytes on day 9 of confluence, replaced with 3 ml of complete medium containing CSTP at the indicated concentrations, and incubated at 37°C for 16 h. The medium was removed, replaced with 1.5 ml of complete medium containing the corresponding concentrations of CSTP, and the cells were incubated at 37°C for 2 h. The medium was removed and passed through a 0.22-μm filter, and lipoprotein lipase activity in the medium was immediately assayed. The plates were rinsed with ice-cold PBS, and the cells were harvested into 1.2 ml of 50 mM NH_3-NH_4Cl buffer (pH 8.2) containing 2% bovine serum albumin and 20 μg/ml heparin, sonicated briefly at 0°C, processed into acetone–ether powders, and assayed for lipoprotein lipase activity. The values given are the mean ± SD of four plates.

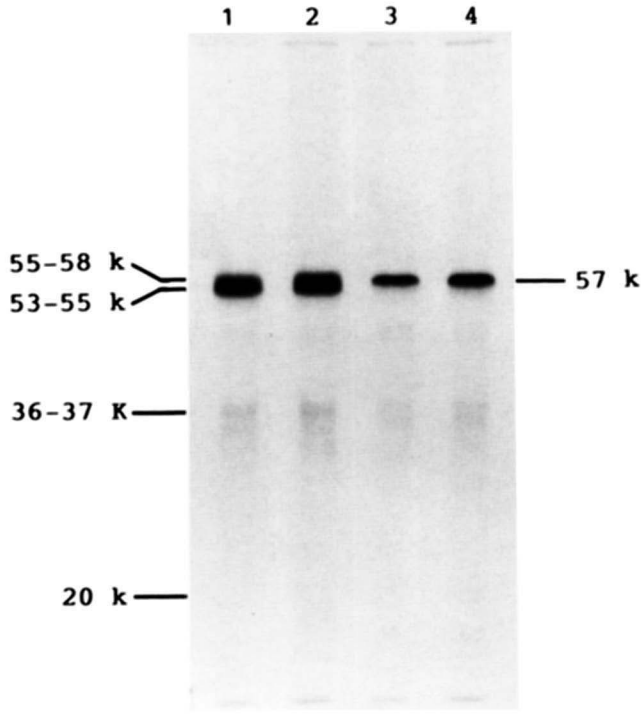

Fig. 1. Autoradiographs demonstrating incorporation of [^{35}S]methionine into lipoprotein lipase by untreated and CSTP-treated 3T3-L1 adipocytes. The culture medium was removed from plates of cells on day 9 of confluence and replaced with 3 ml of complete medium containing 0 or 100 μg/ml CSTP and the cells were incubated for 16 h. Then the cells were incubated for 1 or 2 h with [^{35}S]methionine in methionine-deficient DMEM containing the appropriate additive. ^{35}S-labeled lipoprotein lipase was immunoprecipitated with antiserum to bovine lipoprotein lipase, resolved by SDS-PAGE, and autoradiographed as described in Materials and Methods. The same volume of cell extract was applied to each lane. Lanes 1 and 2: untreated cells incubated with [^{35}S]methionine for 1 and 2 h, respectively; lanes 3 and 4: CSTP-treated cells incubated with [^{35}S]methionine for 1 and 2 h, respectively.

Lipoprotein lipase from untreated cells contained subunits that appeared to migrate as a broad band with M_r = 53,000–58,000 (**Fig. 1**). Pulse-chase experiments showed that this broad band was composed of two groups of subunits, one with M_r = 53,000–55,000 and the other with M_r = 55,000–58,000 (data not shown).

Lipase subunits with M_r = 36,000–37,000 and M_r = 20,000 were also found (Fig. 1), suggesting that lipase was proteolytically cleaved possibly in the lysosome and/or pre-Golgi region of cells during incubation with [^{35}S]methionine, or during incubation of cell extracts with antiserum.

Endo H-digestion studies showed that lipoprotein lipase of untreated cells incubated 2 h with [^{35}S]methionine contained three types of ^{35}S-labeled subunits (**Fig. 2A**). The most prevalent was endo H-resistant subunits with M_r = 55,000–58,000. The group of subunits with M_r = 53,000–55,000 consisted of partially endo H-sensitive

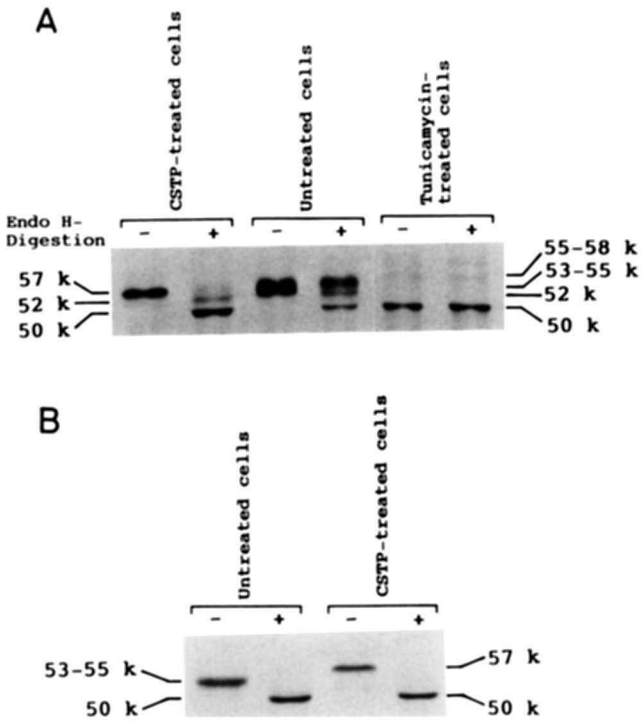

Fig. 2. Digestion with endo H of ^{35}S-labeled lipoprotein lipase subunits extracted from untreated, CSTP-treated, and tunicamycin-treated 3T3-L1 adipocytes. A: Autoradiographs of endo H-digested lipase subunits from cells incubated for 2 h with [^{35}S]methionine. The culture medium was removed from plates of cells on day 10 of confluence and replaced with 3 ml of complete medium containing 0, 100 μg/ml CSTP, or 1 μg/ml tunicamycin, and the cells were incubated for 16 h (untreated and CSTP-treated) or 21.5 h (tunicamycin-treated). ^{35}S-labeled lipoprotein lipase immunoprecipitated from extracts of cells incubated for 2 h with [^{35}S]methionine in methionine-deficient DMEM containing the appropriate additive was solubilized and digested with endo H as described in Materials and Methods. B: Autoradiographs of endo H-digested lipase subunits from cells incubated for 10 min with [^{35}S]methionine. The culture medium was removed from plates of cells on day 8 of confluence and replaced with 3 ml of complete medium containing 0 or 100 μg/ml CSTP and the cells were incubated for 16 h. ^{35}S-labeled lipoprotein lipase immunoprecipitated from extracts of cells incubated (pulsed) for 10 min with [^{35}S]methionine in methionine-deficient DMEM containing the appropriate additive was solubilized and digested with endo H.

subunits yielding a product with M_r = 52,000 and totally endo H-sensitive subunits yielding a product with M_r = 50,000. Totally deglycosylated subunits had the same M_r (50,000) as lipase subunits synthesized in tunicamycin-treated cells (see below) (Fig. 2A).

^{35}S-labeled lipase released into the medium by untreated cells contained subunits with M_r = 55,000–58,000 (**Fig. 3A**). Most of the subunits were endo H-resistant, some were partially endo H-sensitive, and none were totally endo H-sensitive (Fig. 3B).

CSTP-treated 3T3-L1 adipocytes

CSTP, an inhibitor of glucosidase I in endoplasmic reticulum (13–15), decreased the amount of lipoprotein lipase activity in cells in 18 h, and the amount of lipase activity released spontaneously to the medium during the last 2 h of treatment (Table 1). CSTP at 10 and 100 μg/ml decreased cellular lipase activity by 20% and 80%, and decreased release of activity to the medium by 25% and 91%, respectively. The amount of radioactivity in total protein of cells treated with 100 μg/ml CSTP for 18 h and incubated with [^{35}S]methionine during the last 2 h of treatment was 92.5 ± 13.7% of that of untreated cells.

3T3-L1 adipocytes treated with 100 μg/ml CSTP for 18 h and incubated with [^{35}S]-methionine during the last 2 h of treatment contained ^{35}S-labeled lipoprotein lipase composed of subunits that migrated on SDS-PAGE as a narrow band with M_r = 57,000 (Fig. 1).

CSTP-treated cells incubated with [^{35}S]methionine for 10 min contained ^{35}S-labeled lipoprotein lipase composed of only totally endo H-sensitive subunits with M_r = 57,000 (Fig. 2B). Untreated cells incubated with [^{35}S]methionine for the same length of time also contained ^{35}S-labeled lipase composed of only totally endo H-sensitive subunits, but M_r of the subunits in untreated cells (M_r = 53,000–55,000) was 2,000–4,000 less than that in

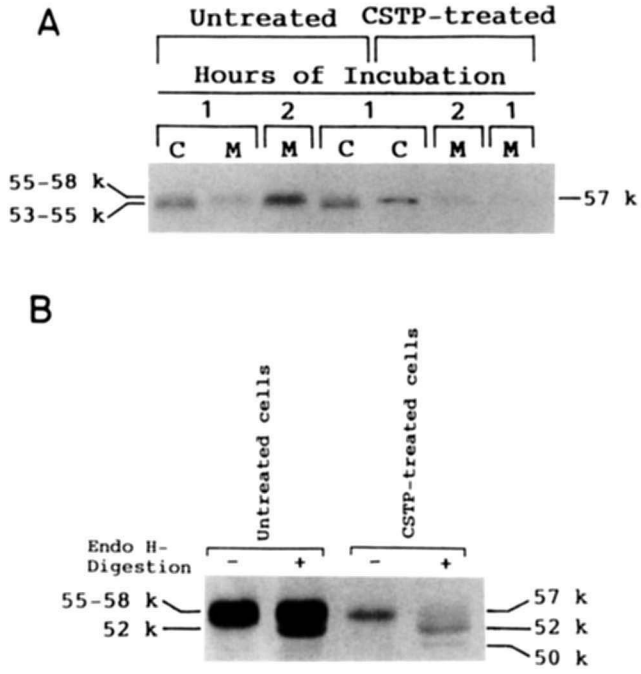

Fig. 3. Release of ^{35}S-labeled lipoprotein lipase into the medium by untreated and CSTP-treated 3T3-L1 adipocytes. ^{35}S-labeled lipoprotein lipase was immunoprecipitated from cells or culture medium of cells incubated for 1 or 2 h with [^{35}S]methionine in the absence or presence of 100 μg/ml CSTP as described in Fig. 2. A: Autoradiographs of ^{35}S-labeled lipoprotein lipase immunoprecipitated from cell extracts or media; C, lipoprotein lipase from cell extracts; M, lipoprotein lipase in medium. B: Autoradiographs of endo H-digested lipase subunits from media of cells incubated for 2 h with [^{35}S]methionine. ^{35}S-labeled lipoprotein lipase immunoprecipitated from the media was solublilized and digested with endo H.

CSTP-treated cells (Fig. 2B). This indicates that trimming of sugar residues from oligosaccharide chains of lipase was impaired in CSTP-treated cells.

^{35}S-labeled lipoprotein lipase in CSTP-treated cells incubated for 2 h with [^{35}S]methionine consisted of three types of subunits. The most prevalent type was totally endo H-sensitive yielding a product with M_r = 50,000, the next was partially endo H-sensitive yielding a product with M_r = 52,000, and a trace was endo H-resistant (Fig. 2A).

CSTP-treated cells released much less ^{35}S-labeled lipoprotein lipase into the medium that did untreated cells (Fig. 3A). The majority of subunits of lipase released by CSTP-treated cells was partially endo H-sensitive, the next was totally endo H-sensitive, and a trace was endo H-resistant (Fig. 3B).

Tunicamycin-treated 3T3-L1 adipocytes

Tunicamycin at 1.0 μg/ml for 24 h completely suppressed lipoprotein lipase activity in cells, confirming previous findings (8, 12). ^{35}S-labeled lipoprotein lipase synthesized during the last 2 h of treatment contained only subunits with M_r = 50,000 (Fig. 2A). These subunits were resistant to endo H-digestion and had the same M_r as deglycosylated subunits of untreated and CSTP-treated cells. These results indicate that lipase subunits with M_r = 50,000 were not glycosylated.

Immunolocalization of lipoprotein lipase in untreated and CSTP-treated 3T3-L1 adipocytes

Lipoprotein lipase was localized in cells with a fluorescent double antibody technique, using chicken antiserum against bovine lipoprotein lipase and rhodamine-labeled affinity-purified rabbit antibodies against chicken IgG (11). Lipoprotein lipase was readily demonstrated in Golgi of untreated 3T3-L1 adipocytes (**Fig. 4A**). This finding is in marked contrast to that in cultured newborn mouse brown adipocytes, in which visualization of immunofluorescent lipase within cells required blocking secretion of lipase with monensin (11, 12).

Treatment of 3T3-L1 adipocytes with 100 μg/ml CSTP for 18 h prevented accumulation of immunofluorescent

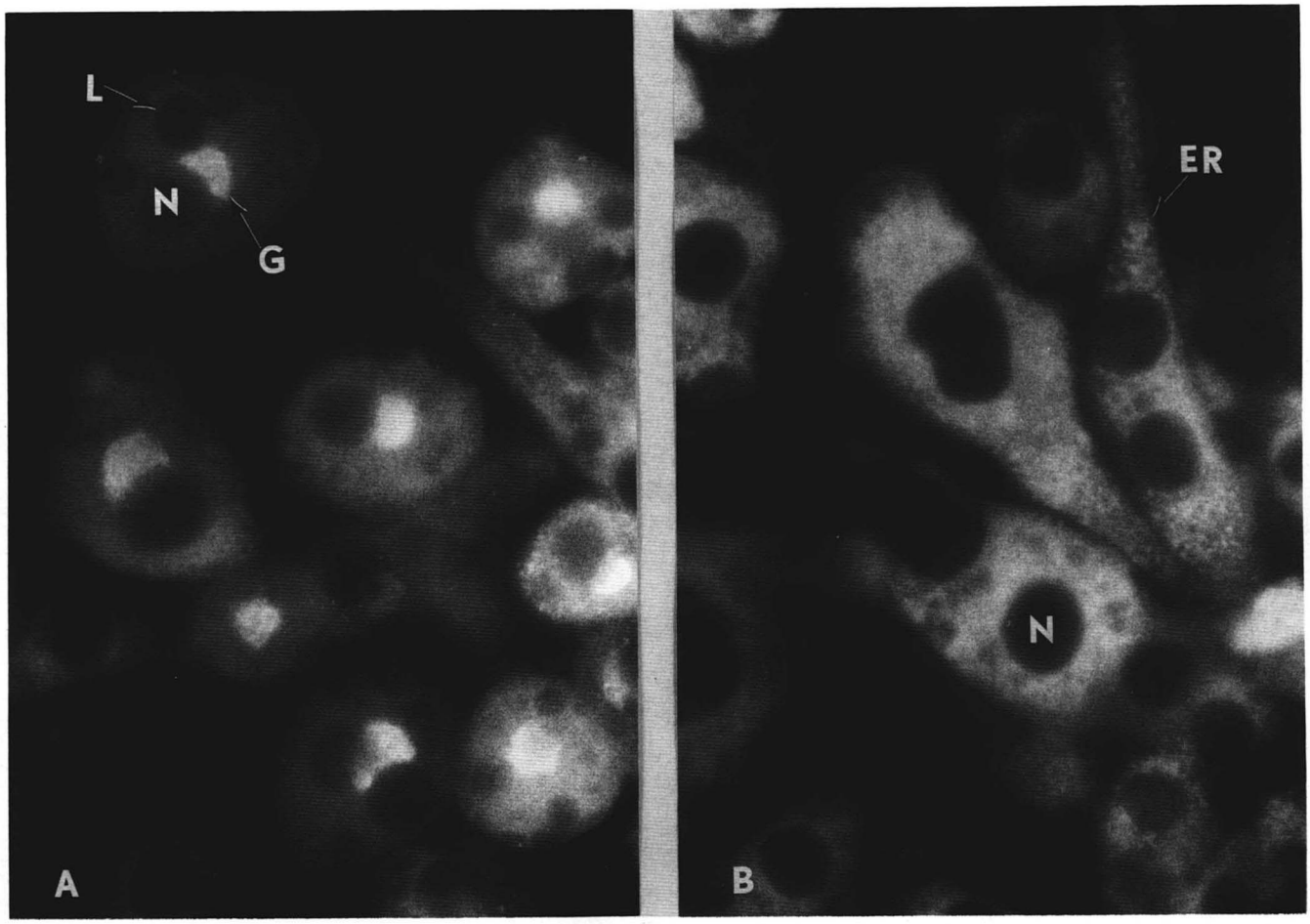

Fig. 4. Light micrographs showing immunofluorescent localization of lipoprotein lipase in untreated and CSTP-treated 3T3-L1 adipocytes. A: Untreated cells; immunofluorescence of lipoprotein lipase is located in the paranuclear Golgi (G) region in untreated cells. B: CSTP-treated cells; immunofluorescence of lipoprotein lipase is distributed in a diffuse reticular/granular pattern, indicating that lipase was located in endoplasmic reticulum (ER), not Golgi, in CSTP-treated cells. L, lipid droplet; N, nucleus; × 600.

lipoprotein lipase in Golgi. The lipase in CSTP-treated cells was distributed in a diffuse reticular/granular pattern, suggesting it was located mostly in endoplasmic reticulum (Fig. 4B). Thus, unprocessed inactive lipase in CSTP-treated cells (Table 1 and Fig. 2) was probably retained in endoplasmic reticulum.

DISCUSSION

We have studied the effect of CSTP, an inhibitor of glucosidase I in endoplasmic reticulum (13–15), on processing, activity, and secretion of lipoprotein lipase in 3T3-L1 adipocytes. Lipoprotein lipase secreted by normal adipocytes contains endo H-resistant (complex) type oligosaccharide chains (Fig. 3B) (9–12). A prerequisite for formation of complex type chains is the removal of glucose residues from oligosaccharide chains in endoplasmic reticulum (21–24). Glucosidase I initiates this process by removing the outermost glucose residue within 2 min after transfer of precursor oligosaccharides to protein (21).

CSTP-treated cells pulsed 10 min with [^{35}S]methionine contained ^{35}S-labeled lipase composed of totally endo H-sensitive subunits that had a M_r 2,000–4,000 larger than that of totally endo H-sensitive subunits in untreated cells (Fig. 2B). This difference suggests that CSTP blocked trimming of sugar residues from both oligosaccharide chains of lipase subunits. The M_r of totally endo H-sensitive lipase subunits in CSTP-treated cells suggests a minimal value of 57,000 for M_r of the murine lipoprotein lipase subunit with two fully glycosylated chains ($Glu_3Man_9GluNAc_2$). This difference also indicates that considerable trimming of the oligosaccharide chains occurred in untreated cells during the first 10 min after glycosylation.

Our immunofluorescent findings showed that lipoprotein lipase accumulated in Golgi in 3T3-L1 adipocytes and that CSTP-treatment impaired transport of lipoprotein lipase from endoplasmic reticulum to Golgi in these cells. Subcellular fraction studies of human hepatoma HepG2 cells demonstrated that 1-deoxynojirimycin, another inhibitor of glucosidase I (25, 26), caused accumulation of endo H-sensitive forms of secretory glycoproteins (α1-antitrypsin and α1-antichymotrypsin) in rough endoplasmic reticulum (27). These findings indicate that the presence of glucose residues on oligosaccharides may impair movement of glycoproteins from endoplasmic reticulum to Golgi.

Although inhibitors of glucosidase I reduce the synthesis of proteins with complex type chains, they never completely block formation of complex type oligosaccharides (13, 15, 27, 28), suggesting that total blockade of glucosidase activity is not possible. However, recent studies demonstrated that several different cells contain an endo-α-D-mannosidase in Golgi that can trim the glucose residues en bloc and adjacent mannose residue from oligosaccharides, and thereby provide a glucosidase-independent pathway for formation of complex N-linked oligosaccharides during glucosidase blockade (22–24). The formation of endo H-resistant lipase subunits in CSTP-treated cells suggests that endo-α-D-mannosidase may be present and active in 3T3-L1 adipocytes.

It is known that the active form of lipoprotein lipase in bovine milk (4, 6), rat adipose tissue and heart (5), and cultured mouse adipocytes (10) is a dimer of identical glycopeptide subunits, and that conversion of the dimeric form to the monomeric form renders the lipase inactive (4–6). But the intracellular site of dimerization of the lipase is not known (12). It is generally thought that oligomerization of proteins occurs in endoplasmic reticulum and is required for transport of such proteins from the reticulum (29). The inhibitory effect of CSTP on activity and intracellular transport of lipoprotein lipase suggests that retention of glucose residues could prevent dimerization and transport of the lipase from endoplasmic reticulum. However, lipoprotein lipase in *cld/cld* adipocytes is also inactive and retained in endoplasmic reticulum (11) in spite of extensive trimming of sugar residues of lipase oligosaccharide chains in endoplasmic reticulum (J-W. Park and R. O. Scow, unpublished experiments). Thus, the lack of dimerization and the retention of lipase in endoplasmic reticulum in CSTP-treated cells may not be due solely to the presence of glucose residues in oligosaccharide chains. The findings suggest that dimerization of lipoprotein lipase may occur after the lipase is transported from endoplasmic reticulum.

These studies show that inhibition of glucosidase I with CSTP blocks trimming of oligosaccharide chains of lipoprotein lipase in endoplasmic reticulum, transport of lipase from endoplasmic reticulum to Golgi, processing of oligosaccharide chains to endo H-resistance, and expression and secretion of lipoprotein lipase activity. The findings indicate that removal of the distal glucose residue from oligosaccharide chains is essential for normal expression of activity and secretion of lipoprotein lipase.

We thank Dr. Thomas Olivecrona, University of Umeå, Umeå, Sweden, for the gift of chicken antiserum against bovine lipoprotein lipase.

Manuscript received 4 February 1992 and in revised form 23 April 1992.

REFERENCES

1. Robinson, D. S. 1970. The function of plasma triglyceride in fatty acid transport. *In* Comprehensive Biochemistry. M. Florkin and E. H. Stotz, editors. Academic Press, New York. **Vol. 18:** 51–116.
2. Scow, R. O., E. J. Blanchette-Mackie, and L. C. Smith. 1976. Role of capillary endothelium in the clearance of chylomicrons: a model for lipid transport from blood by

lateral diffusion in cell membranes. *Circ. Res.* **39:** 149–162.
3. Blanchette-Mackie, E. J., H. Masuno, N. K. Dwyer, T. Olivecrona, and R. O. Scow. 1989. Lipoprotein lipase in myocytes and capillary endothelium of heart: immunocytochemical study. *Am. J. Physiol.* **256:** E818–E828.
4. Olivecrona, T., and G. Bengtsson-Olivecrona. 1987. Lipoprotein lipase from milk—the model enzyme in lipoprotein lipase research. *In* Lipoprotein Lipase. J. Borensztajn, editor, Evener Publishers, Chicago. 15–58.
5. Garfinkel, A. S., E. S. Kempner, O. Ben-Zeev, J. Nikazy, S. J. James, and M. C. Schotz. 1983. Lipoprotein lipase: size of the functional unit determined by radiation inactivation. *J. Lipid Res.* **24:** 775–780.
6. Osborne, J. C., Jr., G. Bengtsson-Olivecrona, N. S. Lee, and T. Olivecrona. 1985. Studies in inactivation of lipoprotein lipase: role of the dimer to monomer dissociation. *Biochemistry.* **24:** 5605–5611.
7. Vannier, C., E-Z. Amri, J. Etienne, R. Negrel, and G. Ailhaud. 1985. Maturation and secretion of lipoprotein lipase in cultured adipose cells. I. Intracellular activation of the enzyme. *J. Biol. Chem.* **260:** 4424–4431.
8. Olivecrona, T., S. S. Chernick, G. Bengtsson-Olivecrona, M. Garrison, and R. O. Scow. 1987. Synthesis and secretion of lipoprotein lipase in 3T3-L1 adipocytes: demonstration of inactive forms of lipase in cells. *J. Biol. Chem.* **262:** 10748–10759.
9. Semb, H., and T. Olivecrona. 1989. The relation between glycosylation and activity of guinea pig lipoprotein lipase. *J. Biol. Chem.* **264:** 4195–4200.
10. 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.
11. Masuno, H., E. J. Blanchette-Mackie, S. S. Chernick, and R. O. Scow. 1990. Synthesis of inactive nonsecretable high mannose-type lipoprotein lipase by cultured brown adipocytes of combined lipase-deficient *cld/cld* mice. *J. Biol. Chem.* **265:** 1628–1638.
12. 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: effect of tunicamycin, monensin, 1-deoxymannojirimycin and swainsonine. *Biochem. J.* **277:** 801–809.
13. Pan, Y. T., H. Hori, R. Saul, B. A. Sanford, R. J. Molyneux, and A. D. Elbein. 1983. Castanospermine inhibits the processing of the oligosaccharide portion of the influenza viral hemagglutinin. *Biochemistry.* **22:** 3975–3984.
14. Palmarczyk, G., and A. D. Elbein. 1985. The effect of castanospermine on the oligosaccharide structures of glycoproteins from lymphoma cell lines. *Biochem. J.* **227:** 795–804.
15. Sasak, V. W., J. M. Ordovas, A. D. Elbein, and R. W. Berninger. 1985. Castanospermine inhibits glucosidase I and glycoprotein secretion in human hepatoma cells. *Biochem. J.* **232:** 759–766.
16. Sekiya, K., H. Okuda, Y. Hotta, and S. Arichi. 1987. Enhancement of adipose differentiation of mouse 3T3-L1 fibroblasts by ginsenosides. *Phytother. Res.* **1:** 58–60.
17. Spooner, P. M., S. S. Chernick, M. Garrison, and R. O. Scow. 1979. Insulin regulation of lipoprotein lipase activity and release in 3T3-L1 adipocytes: separation and dependence of hormonal effects on hexose metabolism and synthesis of RNA and protein. *J. Biol. Chem.* **254:** 10021–10029.
18. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
19. Sakayama, K., H. Masuno, H. Okumura, T. Shibata, and H. Okuda. 1992. Glycosylation of lipoprotein lipase in human subcutaneous and omental adipose tissues. *Biochim. Biophys. Acta.* In press.
20. Hinegardner, R. T. 1971. An improved fluorometric assay for DNA. *Anal. Biochem.* **39:** 197–201.
21. Hubbard, S. C., and R. J. Ivat. 1981. Synthesis and processing of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* **50:** 555–583.
22. Lubas, W. A., and R. G. Spiro. 1988. Evaluation of the role of rat liver Golgi endo-α-D-mannosidase in processing N-linked oligosaccharides. *J. Biol. Chem.* **263:** 3990–3998.
23. Moore, S. E. H., and R. G. Spiro. 1990. Demonstration that Golgi endo-α-D-mannosidase provides a glucosidase-independent pathway for the formation of complex N-linked oligosaccharides of glycoproteins. *J. Biol. Chem.* **265:** 13104–13112.
24. Fujimoto, K., and R. Kornfeld. 1991. α-Glucosidase II-deficient cells use endo α-mannosidase as a bypass route for N-linked oligosaccharide processing. *J. Biol. Chem.* **266:** 3571–3578.
25. Saunier, B., R. K. Kilker, Jr., J. S. Tkacz, A. Quaroni, and A. Herscovics. 1982. Inhibition of N-linked complex oligosaccharide formation by 1-deoxynojirimycin, an inhibitor of processing glucosidases. *J. Biol. Chem.* **257:** 14155–14161.
26. Gross, V., T. Andus, T-A. Tran-Thi, R. T. Schwarz, K. Decker, and P. C. Heinrich. 1983. 1-Deoxynojirimycin impairs oligosaccharide processing of α_1-proteinase inhibitor and inhibits its secretion in primary cultures of rat hepatocytes. *J. Biol. Chem.* **258:** 12203–12209.
27. Lodish, H. F., and N. Kong. 1984. Glucose removal from N-linked oligosaccharides is required for efficient maturation of certain secretory glycoproteins from the rough endoplasmic reticulum to the Golgi complex. *J. Cell Biol.* **98:** 1720–1729.
28. Gross, V., T-A. Tran-Thi, R. T. Schwarz, A. D. Elbein, K. Decker, and P. C. Heinrich. 1986. Different effects of the glucosidase inhibitors 1-deoxynojirimycin, N-methyl-1-deoxynojirimycin and castanospermine on the glycosylation of rat α_1-proteinase inhibitor and α_1-acid glycoprotein. *Biochem. J.* **236:** 853–860.
29. Rose, J. K., and R. W. Doms. 1988. Regulation of protein export from the endoplasmic reticulum. *Annu. Rev. Cell. Biol.* **4:** 257–288.