Asparagine-linked Oligosaccharides Protect Lamp-1 and Lamp-2 from Intracellular Proteolysis*

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Lysosomes contain several integral membrane proteins (termed Lamps and Limps) that are extensively glycosylated with asparagine-linked oligosaccharides. It has been postulated that these glycans protect the underlying polypeptides from the proteolytic environment of the lysosome. Previous attempts to test this hypothesis have been inconclusive because they utilized approaches that prevent initial glycosylation and thereby impair protein folding. We have used endoglycosidase H to remove the Asn-linked glycans from fully folded lysosomal membrane proteins in living cells. Deglycosylation of Lamp-1 and Lamp-2 resulted in their rapid degradation, whereas Lamp-2 was relatively stable in the lysosome in the absence of high mannose Asn-linked oligosaccharides. Depletion of Lamp-1 and Lamp-2 had no measurable effect on endosomal/lysosomal pH, osmotic stability, or density, and cell viability was maintained. Transport of endocytosed material to dense lysosomes was delayed in endoglycosidase H treated cells, but the rate of degradation of internalized bovine serum albumin was unchanged.

These data provide direct evidence that Asn-linked oligosaccharides protect a subset of lysosomal membrane proteins from proteolytic digestion in intact cells.

One of the proposed functions of asparagine-linked oligosaccharides is to protect the underlying polypeptide from proteolysis (1). The strongest evidence for this conclusion comes from in vitro studies that utilize mature glycoproteins that are treated with various glycosidases to remove their carbohydrate units. By comparing glycosylated proteins with their unglycosylated counterparts, it has been shown that glycosylation increases thermal stability, solubility, dynamic stability, and resistance to protease digestion (2–5). In contrast, experiments designed to analyze the importance of glycosylation in protecting endogenous proteins from proteolysis in living cells have used approaches that prevent the initial glycosylation of the nascent protein. Most of these studies have used tunicamycin, an inhibitor of UDP-GlcNAc:dolichyl-phosphate GlcNAc-1-phosphate transferase, to produce unglycosylated proteins in vivo. However, the inhibition of initial glycosylation disrupts protein folding to such an extent that it induces the “unfolded protein response,” a complex stress response characterized by the up-regulation of several chaperones in the endoplasmic reticulum (6). Many of the misfolded proteins are rapidly degraded in the endoplasmic reticulum (7). Therefore, a decrease in the half-life of a protein synthesized in the presence of tunicamycin usually reflects endoplasmic reticulum-mediated proteolysis of misfolded proteins. The use of site-directed mutagenesis to remove Asn-linked glycosylation signals has provided a means for exploring the role of individual glycosylation sites on the behavior of the protein, but potential problems with proper protein folding remain.

To avoid these difficulties, we sought a method for removing Asn-linked glycans from mature proteins in vivo, thereby bypassing any effects on initial protein folding. In this paper, we describe such a method based on the ability of endoglycosidase H (endo H)1 to cleave high mannose oligosaccharides from fully folded proteins. Using this method, we have examined the role of glycosylation in protecting lysosomal membrane proteins from degradation. Of all intracellular proteins, the components of the lysosome exist in the most proteolytic environment. The integral membrane proteins that are major constituents of the lysosomal membrane are extensively glycosylated and include Lamp-1 and Lamp-2 (lysosome-associated membrane proteins) and Lamp-1 and Lamp-2 (lysosomal integral membrane proteins) (for a review, see Ref. 8). Indeed, the majority of the weight of these molecules is derived from carbohydrates (9–11). As such, these proteins make excellent models to test the hypothesis that core glycosylation protects against proteolysis in vivo. Using endo H to deglycosylate the various lysosomal membrane proteins in intact cells, we have determined that Asn-linked high mannose glycans protect Lamp-1 and Lamp-2 from degradation but are not essential for the prolonged survival of Lamp-2.

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—All chemicals were analytical grade and obtained from Sigma except for Percoll (Amersham Pharmacia Biotech), ECL reagents including horseradish peroxidase-conjugated secondary antibodies and Na125I (Amersham Pharmacia Biotech), fluorophore-conjugated secondary antibodies (Jackson Immunoresearch), nitrocellulose (Schleicher and Schuell), 1-deoxymannojirimycin (Oxford Glycosystems), IODO-BEADS (Pierce), fluorescein isothiocyanate-labeled dextran (FITC-dextran) (Molecular Probes, Inc., Eugene, OR), and endo H and endo H1 (New England Biolabs). β-Glucuronidase was purified from the secretion of 13.2.1 mouse L cells and iodinated as described previously (12). This cell line, which has been engineered to secrete large amounts of β-glucuronidase, was provided by William Sly (St. Louis University). Mouse monoclonal antibodies UH1 (anti-CHO Lamp-1) and UH3 (anti-CHO Lamp-2) developed by Dr. Thomas August (Johs Hopkins) were from the Developmental Studies Hybridoma Bank (University of Iowa). Mouse monoclonal E10D10 (anti-CHO Lamp-2) (Affinity Bioreagents), ESD9

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1 The abbreviations used are: endo H, endoglycosidase H; CHO, Chinese hamster ovary; NRK, normal rat kidney; DMJ, 1-deoxymannojirimycin; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; Man-6-P/IGF-II receptor, mannose 6-phosphate/insulin-like growth factor II receptor; FITC, fluorescein isothiocyanate.
(anti-CHO Lamp-2), and affinity-purified rabbit anti-canine Rab7 were generous gifts of S. Pfeffer (Stanford) (13–15). Affinity-purified rabbit anti-human Lamp-1 and mouse monoclonal 10D10 (anti-rat Lamp-2) were generous gifts of M. Fukuda (Burnham Institute) (16) and M. Viola (Facultes Universitaires Notre Dame De La Paix, Belgium) (17), respectively. Mouse monoclonal antibodies against rat Limp-2 were provided by I. Sandalov (University of Madrid, Spain) (10) and K. Akasaki (Fukuyma University, Japan) (18). Affinity-purified goat anti-human β-glucuronidase and affinity-purified rabbit anti-bovine Man-6-P/Gl/GFII receptor antibodies were provided by W. Sly and J. Traub (Washington University), respectively.

CHO-β-Glucuronidase, CHO 15B cells deficient in N-acetylglucosaminyltransferase I have been previously described (19).

Normal rat kidney (NRK) cells were obtained from ATCC. The cells were grown in α-minimal essential medium containing 10% fetal calf serum in a 37 °C incubator supplemented with 5% CO₂. The NRK cells were treated with 1 mM 1-deoxymannojirimycin (DMJ) for at least 3 days prior to use in the experiments. The DMJ-containing medium was replaced daily.

Endo H Treatment of Intact Cells—CHO 15B or DMJ-treated NRK cells were grown in six-well plates (22-mm diameter) to 70% confluency in complete medium containing 20 milliunits/ml endo H for the indicated number of hours. Cells were washed, scraped in PBS, and recovered by centrifugation, and the cell pellet was solubilized in reducing SDS-PAGE sample buffer. Aliquots were subjected to SDS-PAGE and immunoblotting with various antibodies as described in the figure legends. In some experiments, the protease inhibitors pepstatin A (1 μM) and leupeptin (1 μM) were added simultaneously with endo H. To examine the effect of the endo H on a soluble intracellular protein, CHO 15B cells were allowed to internalize purified human β-glucuronidase for 12 h and then chased for 2 h prior to the addition of the endo H.

In Vitro Endo H Digestion—CHO 15B and DMJ-treated NRK cell pellets were solubilized with 1% Triton X-100 in PBS, and 50-μg aliquots were digested with 0, 0.1, 0.5, or 2.5 milliunits of endo H in PBS, pH 7.4, containing 1 μM pepstatin A and 1 μM leupeptin for 30 min at 37 °C. The reaction was stopped by the addition of SDS-PAGE sample buffer and boiling. Following SDS-PAGE, the CHO 15B extracts were blotted for Lamp-1, and the NRK extracts were blotted for Limp-2.

Electrophoresis and Immunoblotting—Discontinuous SDS-PAGE and protein transfer onto nitrocellulose were performed using the BioRad Mini-Protean II and Trans-blot apparatuses according to the manufacturer’s instructions. Buffer systems were those of Laemmli and Towbin, respectively (20, 21). Membranes with transferred proteins were blocked in 5% skim milk in PBS containing 0.1% Tween 20 (PBST) for 1 h and then incubated with the specified antibodies, as detailed in the figure legends, for 1 h with constant shaking. All antibodies were diluted in PBST containing 3% milk. After washing the blots with PBST, the membranes were incubated with the horseradish peroxidase-conjugated secondary antibody for 1 h and washed in PBST. The ECL reaction was for 1 min as recommended by the manufacturer, and the chemiluminescent signals were visualized on autoradiographic film. For quantitative analysis, autoradiographs were analyzed using a Personal Densitometer (Molecular Dynamics, Inc., Sunnyvale, CA) equipped with ImageQuant software.

Determination of Endosomal/Lysosomal pH—The pH of the endosomal/lysosomal system was determined as described by Ohkuma and Poole with modifications (22).

Percoll Gradient Fractionation—The method of Percoll gradient fractionation has been described previously (24).

Transport of 125I-Labeled β-Glucuronidase to Dense Lysosomes—CHO 15B cells were grown in 10-cm plates to 70% confluency and incubated in complete medium in the presence or absence of 20 milliunits/ml endo H for 18 h. Cells were then washed with PBS and incubated in complete medium containing 3 × 10⁶ cpm of 125I-labeled β-glucuronidase for 15 min. Residual radiolabeled ligand was removed by three washes with complete medium, and the cells were chased for 30 or 60 min in complete medium. Cells were homogenized, and subcellular organelles were fractionated on 18% Percoll gradients as described (24). Nine 1-ml fractions were collected from the bottom of the gradient, which was grayed for β-glucuronidase activity, and the remainder was counted in a γ-counter.

Immunofluorescence—NRK cells were grown on coverslips in complete media and treated with 20 milliunits/ml endo H for greater than 12 h. Cells were washed with PBS, fixed for 30 min in 3% paraformaldehyde in PBS; permeabilized in PBS containing 5% donkey sera, 5% goat serum, 0.5% bovine serum albumin, and 0.2% saponin for 30 min; washed with PBS; and double-labeled with primary antibodies as described in the figure legends. Secondary antibodies were Cy3-conjugated donkey anti-rabbit IgG and FITC-conjugated goat anti-mouse IgG. Slides were examined by using a Nikon epifluorescence microscope.

Acridine Orange Staining—Cells were grown on coverslips and treated with 20 milliunits/ml endo H as described above. Acridine Orange was then added to the medium for 5 min, and the cells were immediately photographed.

RESULTS

Endoglycosidase H Removes the Asn-linked Oligosaccharides of Lysosomal Membrane Proteins of Intact Cells—We have addressed the role of glycosylation of lysosomal membrane proteins at the level of the endosomal/lysosomal system by removing the Asn-linked oligosaccharides from mature proteins in living cells using a novel method based on the activity of endo H. This enzyme cleaves at the chitobiose core of Asn-linked high mannose and hybrid oligosaccharides, leaving behind a single N-acetylglucosamine (we will refer to this as deglycosylation). Because endo H is incapable of cleaving processed, complex-type oligosaccharides, we adopted two complementary strategies to prevent the formation of such structures in the cells under investigation. In the first strategy, we utilized a ricin-resistant CHO cell line (CHO 15B) deficient in UDP-N-acetylglucosamine:glycoprotein N-acetylglucosaminyltransferase I activity (19, 25). This enzyme is necessary for processing Man₃,₃-GlcNAc₂ to complex-type structures, and consequently the Asn-linked oligosaccharides in the cell line remain as high mannose forms (Man₃,₃-GlcNAc₂), which are endo H substrates (26). The second strategy employed the Golgi α-mannosidase I inhibitor DMJ (27). NRK cells grown in the presence of 1 mM DMJ for 3 days contain glycoproteins with high mannose oligosaccharides that are cleavable by endo H.

In the initial experiments, 20 milliunits/ml endo H was added to the media of CHO 15B cells or DMJ-treated NRK cells. After various times of incubation, cell lysates were prepared, and the state of the lysosomal membrane proteins was analyzed by immunoblotting. By 3 h of endo H treatment, a decrease in the molecular weight of the lysosomal glycoproteins Lamp-1, Lamp-2, and Limp-2 was observed, consistent with the removal of multiple Asn-linked high mannose oligosaccharides (Fig. 1). Interestingly, in both CHO 15B cells (A) and DMJ-treated NRK cells (B) the decline in the molecular weight of Lamp-1 and Limp-2 was associated with a significant loss of immunoreactivity on the immunoblots. This decline continued such that almost all of the Lamp-1 immunoreactivity was lost by 18 h, and the Limp-2 disappeared by 6–9 h.

The rate of loss of deglycosylated Lamp-2 was slowed by the addition of the lysosomal protease inhibitors pepstatin A and leupeptin to the incubation medium, consistent with the loss being due to proteolysis, most likely in the lysosome (Fig. 2). However, the protease inhibitors were least effective at protecting the lowest molecular weight (most deglycosylated) forms of Lamp-2. This observation emphasizes the susceptibility of the Lamp-2 protein core to proteolysis in the absence of a threshold level of glycosylation. Deglycosylated Lamp-1 was also partially protected in the presence of leupeptin and pepstatin A (data not shown). These findings directly demonstrate the importance of the multiple Asn-linked glycans of Lamp-1 and Lamp-2 for protecting the protein core from digestion in the proteolytic environment of the lysosome.

The molecular weight of Limp-2 was also reduced after DMJ-treated NRK cells were incubated in medium containing endo H (Fig. 1C). However, in contrast to the findings with Lamp-1 and Lamp-2, Limp-2 was relatively stable in the lysosomal environment in the absence of high mannose oligosaccharides as demonstrated by the minor reduction in Limp-2 protein.
observed with 48 h of endo H treatment. To confirm that all of the Asn-linked glycosylated native Limp-2 are accessible to endo H, DMJ-treated NRK cells that had been incubated in the presence of endo H for 30 h were solubilized in 1% Triton X-100, and the proteins were denatured by boiling in 0.5% SDS, 1% β-mercaptoethanol followed by redigestion with endo H. As shown in Fig. 3B, the redigested Limp-2 migrated at the same position on SDS-PAGE as the material originally recovered from the endo H-treated cells. This indicated that endo H releases all the high mannose units from native Limp-2.

**Endoglycosidase H Functions in Intracellular Compartment**

Since the endo H was added to the medium of living cells, it could encounter the various lysosomal membrane proteins when they traffic to the cell surface (28). Alternatively, the endo H could be internalized by pinocytosis and act at the level of the endosomal/lysosomal vesicular compartments. To distinguish these two possibilities, we first incubated Triton X-100 lysates of CHO 15B and DMJ-treated NRK cells with various concentrations of endo H for 30 min and then determined the extent of deglycosylation of Lamp-1 and Limp-2 (Fig. 3A). It is apparent that both proteins are significantly deglycosylated by 0.1 milliunit of endo H and fully deglycosylated by 2.5 milliunits. Thus, if the living cells took up just a few percent of the 20 milliunits/ml endo H from the medium over the 3–6-h period of the standard experiments, there would probably be sufficient intracellular endo H to deglycosylate the lysosomal membrane glycoproteins, assuming that the endo H remained active in the proteolytic environment of the endosomal/lysosomal system.

To test for intracellular endoglycosidase activity, we examined the effect of endo H treatment on an intracellular pool of β-glucuronidase, a soluble lysosomal glycoprotein. CHO 15B cells were allowed to internalize β-glucuronidase for 12 h and then were incubated with 20 milliunits/ml endo H for various times. As demonstrated in Fig. 4, the entire pool of internalized β-glucuronidase was deglycosylated after 6 h of endo H treatment (lane d). No endo H cleavage was detected when cells were incubated briefly (15 min) with the glycosidase and collected in its presence, thereby excluding the possibility that deglycosylation occurred during processing of the sample (lane e). Since none of the β-glucuronidase recovered in the lysates could have cycled to the cell surface (because endo H would have removed the oligosaccharide bearing the mannose 6-phosphate tag and thus prevented reinternalization), these observations indicate that at least a fraction of the endo H was internalized by the cells.

**Lamp-1 and Limp-2 Are Restored following Removal of the Endo H**—After 18 h of endo H treatment, greater than 95% of total Lamp-1 and Limp-2 was degraded. Since vacuolation of the cytoplasm was observed in treated cells (see below), we considered the possibility that prolonged exposure to the endoglycosidase resulted in irreversible cellular destruction. In an attempt to address this issue, DMJ-treated NRK cells were incubated in the presence of endo H for 24 h to deplete Lamps and then allowed to recover for 24 h following the removal of...
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**FIG. 4.** Endoglycosidase H acts intracellularly. CHO 15B cells were incubated with 6.4 units of purified human β-glucuronidase for 12 h to allow internalization and then incubated in the absence (lane c) or presence of 20 milliunits/ml endo H for 6 h (lane d) or 15 min (lane e). Cells were processed for immunodetection of β-glucuronidase as described in Fig. 1. Lane b contained 50 milliunits of purified β-glucuronidase, and lane a contained 50 milliunits of β-glucuronidase that had been partially digested with endo H in vitro. Note that the internalized β-glucuronidase gets proteolytically processed, hence the intracellular form is smaller (lane c versus lane b).

The endo H from the medium. As shown by the immunoblots in Fig. 5, the level of Lamp-1 protein went from undetectable after endo H treatment (lane c) to nearly normal levels (compare lane f with lane c) after the 24-h chase in the absence of endo H. This finding indicates that the cells are viable and that the Lamp-1 biosynthetic pathway is intact after the prolonged endo H treatment. The rate at which Lamp-1 was replenished is consistent with the documented half-life of this protein (9, 10). Similar observations were made with Lamp-2 (data not shown).

Since the length of endo H treatment required to deplete Lamps does not affect cell viability, we used this method to investigate possible lysosomal functions of Lamp-1 and Lamp-2.

**Lamps Are Not Required for Acidification of the Endosomal/Lysosomal System**—Lamp-1 and Lamp-2 have been estimated to constitute about 12% of the proteins in the lysosomal membrane (29). This density of Lamps, in conjunction with their extensive glycosylation, has been suggested to form a carbohydrate barrier to the hydrolytic lysosomal lumen that would protect other proteins embedded in the lysosomal membrane (e.g., components of the vacuolar H⁺-ATPase), as well as the membrane itself (8, 9). To test the possibility that Lamps prevent degradative damage of the proton pump, we assessed the function of this macromolecular complex under conditions of Lamp depletion. The combined endosomal and lysosomal pH in living cells was measured using established methods based on the pH-dependent signals from FITC-dextran (22). In these experiments, CHO 15B cells were allowed to internalize FITC-dextran for 15 h and then were incubated in the absence or presence of 20 milliunits/ml endo H to deplete the Lamps. Under these conditions, the endosomal/lysosomal pH was determined to be 5.4 ± 0.2 for untreated cells and 5.3 ± 0.16 for endo H-treated cells (Fig. 6). These findings indicate that the deglycosylation and subsequent depletion of Lamps have no significant effect on the endosomal/lysosomal pH. The activity of the proton pump was further tested by measuring the pH change induced by the addition of 10 mM NH₄Cl and the rate and degree of acidification after washing out this weak base. After removal from the NH₄Cl solution, the endosomal/lysosomal pH rebounded with identical kinetics to initial levels in both endo H-treated and untreated cells, which demonstrates the presence of an active proton pump. Therefore, we conclude that glycosylated Lamp-1 and Lamp-2 are not vital for vacuolar H⁺-ATPase function.

**Dense Lysosomes Are Present in Lamp-depleted Cells**—Lyso- somes are characterized not only by the presence of acid hydrolases but also by the organelle’s high density. We considered the possibility that Lamp-1 and Lamp-2 might be required for the formation of these dense structures. To address this, we subjected cell homogenates of untreated and endo H-treated cells to subcellular fractionation on 18% Percoll gradients. Under these conditions, dense lysosomes are recovered in the...
bottom third of the gradient, whereas lighter organelles, including endosomes, are found in the upper third of the gradient. As shown in Fig. 7, the distribution of Limp-2 in the gradient was identical in the control and Lamp-depleted DMJ-treated NRK cells. This establishes that Lamp-1 and Lamp-2 are not necessary for the formation of dense lysosomes. However, the dense lysosome fraction of the Lamp-depleted cells had a 30–60% decrease in the proportion of total β-hexosaminidase activity recovered in the gradients (Fig. 7). A similar decrease in the percentage of β-glucuronidase activity in the dense lysosome fraction was also observed in endo H-treated cells (data not shown). In both CHO and NRK cells, treatment with endo H did not result in a decrease in the total activity of these two acid hydrolases, although the β-glucuronidase was documented to be deglycosylated (Fig. 4).

The finding that the distribution of the soluble acid hydrolases differs from that of Limp-2 in the gradient is consistent with an alteration in the trafficking of the acid hydrolases, particularly in their transport to dense lysosomes. Direct evidence for this possibility was obtained by monitoring the arrival of endocytosed $^{125}$I-labeled β-glucuronidase in dense lysosomes. In this experiment, control and Lamp-depleted CHO 15B cells were allowed to internalize $^{125}$I-labeled β-glucuronidase for 15 min, and following a chase period of 30 or 60 min, the cells were homogenized and subjected to subcellular fractionation on 18% Percoll gradients. The distribution of the $^{125}$I-labeled β-glucuronidase was determined, and the percentage recovered in the dense lysosome fraction was calculated (Fig. 8). It is apparent that the transport of β-glucuronidase to the dense lysosomes is slower in the endo H-treated cells, although it eventually reaches that organelle. This result is consistent with there being a partial block in the transport of material between endosomes and dense lysosomes and helps explain the observed alteration in the steady state distribution of the acid hydrolases.

The Degradative Function of the Endosomal/Lysosomal System Is Intact in Lamp-depleted Cells—We next compared the ability of control and endo H-treated CHO 15B cells to degrade $^{125}$I-labeled phosphopentamannosylated bovine serum albumin that had been internalized via the Man-6-P/IGF-II receptor. The rate of proteolysis was very similar in both cell types, demonstrating that the Lamp-depleted cells maintained the proteolytic function of the endosomal/lysosomal system (data not shown). In view of the lag in transporting β-glucuronidase to dense lysosomes in the endo H-treated cells, it would appear that at least a portion of the proteolysis is occurring in endosomal compartments proximal to the dense lysosomes. This is consistent with the conclusion of
The data presented in this report establish that endo H can be used to excise the Asn-linked glycans from mature proteins in intact cells, thereby allowing the analysis of the biologic roles of these structures. The major advantage of this technique over approaches that prevent initial glycosylation is that the nascent protein is allowed to fold properly and traffic through the biosynthetic pathway prior to the removal of its oligosaccharide units. This is quite important, since many glycoproteins misfold and are subsequently degraded when Asn-linked glycosylation is prevented. On the other hand, there are several limitations to the endo H technique. The first is that it can only be used on cells that are unable to process high mannose oligosaccharides to complex-type glycans. We used two sources of cells to meet this criterion. The first was a ricin-resistant CHO cell line (15B) that is deficient in N-acetylglucosaminyltransferase I (19). This inhibitor blocks the processing of Man$_{8-9}$GlcNAc$_2$ oligosaccharides to the Man$_{9}$GlcNAc$_2$ intermediate and thereby keeps the Asn-linked glycans in an endo H-sensitive state. This approach can be used with virtually any cell line that grows in vitro. Another limitation is that the endo H cleaves the Asn-linked glycans from all of the glycoproteins of the cell surface and the endosomal/lysosomal system. While this is not a problem when cellular functions remain intact following endo H treatment, as most do, it prevents specific proteins from being implicated in processes that become abnormal.

The specific biological question we have addressed is whether Asn-linked glycans protect the underlying protein from proteases in intact cells. We chose to examine the lysosomal membrane glycoproteins because they are highly glycosylated and must exist in a very proteolytic environment. These type I membrane proteins have large luminal domains of 380–396 amino acids containing 16–20 sites for Asn-linked oligosaccharide addition, most of which are used (9–11). Normally, the majority of the oligosaccharides are processed to complex-type structures that contain repeating lactosamine units and a high content of sialic acid (16). However, the absence of polylactosamine units has no appreciable effect on the half-life of Lamps or lysosomal function in CHO cells (33). While it had been speculated that the high density of carbohydrates on these molecules protects them from proteolysis, this issue had not been directly addressed prior to our study.

We find that the Asn-linked oligosaccharides of Lamp-1, Lamp-2, and Lamp-2 are readily removed simply by incubating cells in the presence of endo H for 3–6 h. Since the high mannose units of at least some soluble acid hydrolases are also excised, it seems most likely that the endo H is entering the endosomal/lysosomal system via pinocytosis and is acting at these intracellular sites. However, we cannot exclude the possibility that the Lamps are cycling to the cell surface, where they encounter the endo H (28). Regardless of the site of glycan removal, the critical finding is that deglycosylated Lamp-1 and Lamp-2 are rapidly degraded by proteases in their resident environment. This establishes that Asn-linked glycans, even of the high mannose type, protect these two membrane glycoproteins from the action of proteases. A previous study demonstrated that Lamp-1 synthesized in the presence of tunicamycin is rapidly degraded, but the interpretation of this finding is confounded by the fact that the Lamp-1 may have misfolded in the endoplasmic reticulum and been recognized as an abnormal protein by the quality control system (10).

In contrast to the rapid degradation of deglycosylated Lamp-1 and Lamp-2, Lamp-2 remained long lived in the absence of its Asn-linked high mannose glycans. This result differs from the finding that Lamp-2 synthesized in the presence of
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transfer of materials between late endosomes and dense lysosomes. The appearance of this structure in endo H-treated cells may reflect a defect in the fission process and can account for the slowed rate of transport of β-glucuronidase to dense lysosomes and the shift in the steady state distribution of the acid hydrolases on Percoll gradients. However, further investigation will be required to determine if the appearance of this compartment is the result of Lamp-1 and Lamp-2 depletion or the consequence of the loss of other glycoproteins.

While this manuscript was in preparation, Andrejewski et al. (41) reported their findings with Lamp-1-deficient mice. The mice were viable and fertile and exhibited minimal histologic abnormalities, primarily a mild astrogliosis in a limited region of the brain. Lysosomal properties, including pH, osmotic stability, density, shape, enzyme content, and subcellular distribution were intact. The authors noted that in several tissues Lamp-2 was up-regulated, possibly compensating for the loss of Lamp-1. However Lamp-2 was not up-regulated in the liver nor in fibroblasts derived from Lamp-1-deficient mice. Further, the brain, which had only a mild astrogliosis, contains almost no Lamp-2. These results are consistent with our finding that Lamp-1 and Lamp-2 depletion has minimal effects on lysosomal function. The authors quote unpublished data that the loss of both Lamp-1 and Lamp-2 results in minimal effects on embryonic lethality. This indicates a role of the Lamp in development that is not required for the viability of cells grown in tissue culture.

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REFERENCES

1. Varki, A. (1993) Glyobiology 3, 97–130
2. Stocek, U., Cramer, M., and Mannherz, H. G. (1992) Biochim. Biophys. Acta 1122, 327–332
3. Ruhli, M., Stachel, J., and Mannherz, H. G. (1994) Biochem. Biophys. Acta 1204, 117–124
4. van Berkel, P. H., Geerts, M., and Geuze, H. J. (1995) Biochem. J. 312, 107–114
5. Ruhli, M., Stachel, J., and Mannherz, H. G. (1994) Biochem. Biophys. Acta 1204, 117–124
6. Brewer, J. W., Cleveland, J. L., and Hendershot, L. M. (1997) EMBO J. 16, 7207–7216
7. Siira, J., Chapman, R., and Walter, P. (1998) Trends Cell Biol. 8, 245–249
8. Hunziker, W., and Geuze, H. J. (1996) BioEssays 18, 379–389
9. Lewis, V., Green, S. A., Marsh, M., Vihko, P., Helenius, A., and Mellman, I. (1985) J. Cell Biol. 100, 1839–1847
10. Barriocanal, J. G., Bonifacino, J. S., Yuan, L., and Sandoval, I. V. (1986) J. Biol. Chem. 261, 16755–16763
11. High, M., Saurina, L., Bainton, D. F., Holt, V. K., Cha, Y., Hildreth, J. E., and August, J. T. (1989) Arch. Biochem. Biophys. 268, 360–378
12. Jaffé, L., Manfredi, W. M., Gregory, W., and Kornfeld, S. (1999) J. Biol. Chem. 274, 11068–11077
13. Lombardi, D., Soldati, T., Riederer, M. A., Goda, Y., Zerial, M., and Pfeffer, S. R. (1999) EMBO J. 18, 677–682
14. Soldati, T., Rancano, C., Geissler, H., and Pfeffer, S. R. (1995) J. Cell Biol. 116, 18911–18919
15. Riederer, M. A., Soldati, T., Shapiro, A. D., Lin, J., and Pfeffer, S. R. (1994) J. Cell Biol. 125, 573–582
16. Carlsson, S. R., Roth, J., Piller, F., and Fukuoda, M. (1988) J. Biol. Chem. 263, 9013–9019
17. Jaffé, L., Dubois, F., Watanabe-De Coninck, S., and Watanabe, K. (1995) Eur. J. Biochem. 249, 862–869
18. Akatsuk, K., Michihara, A., Fukuwama, M., Kinosita, H., and Tsuji, H. (1994) J. Biochem. (Tokyo) 116, 670–676
19. Gottlieb, C., Skinner, A. M., and Kornfeld, S. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 1078–1082
20. Laemmli, U. K. (1970) Nature 227, 680–685
21. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
22. Ohkuma, S., and Poole, B. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 3327–3331
23. Bals, E., and Rothman, J. E. (1985) Arch. Biochem. Biophys. 240, 413–425
24. Rohrer, J., Schweizer, A., Johnson, K. F., and Kornfeld, S. (1995) J. Cell Biol. 129, 1297–1306
25. Gottlieb, C., Baenziger, J., and Kornfeld, S. (1975) J. Biol. Chem. 250, 3303–3309
26. Kornfeld, R., and Kornfeld, S. (1985) Annu. Rev. Biochem. 54, 631–664
27. Kornfeld, R., and Kornfeld, S. (1984) Biochem. Biophys. Res. Commun. 125, 326–331
28. Lippincott-Schwartz, J., and Fambrough, D. (1987) Cell 49, 669–677
29. Fukuda, M., Vitala, J., Matteson, J., and Carlsson, S. R. (1988) J. Biol. Chem. 263, 1078–1082
30. Laemmli, U. K. (1970) Nature 227, 680–685
31. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
32. Ohkuma, S., and Poole, B. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 3327–3331
33. Bals, E., and Rothman, J. E. (1985) Arch. Biochem. Biophys. 240, 413–425
34. Rohrer, J., Schweizer, A., Johnson, K. F., and Kornfeld, S. (1995) J. Cell Biol. 129, 1297–1306
35. Gottlieb, C., Baenziger, J., and Kornfeld, S. (1975) J. Biol. Chem. 250, 3303–3309
36. Kornfeld, R., and Kornfeld, S. (1985) Annu. Rev. Biochem. 54, 631–664
37. Fukuda, M., Vitala, J., Matteson, J., and Carlsson, S. R. (1988) J. Biol. Chem.
Deglycosylated Lamps Are Rapidly Degraded

30. Tjelle, T. E., Brech, A., Juvet, L. K., Griffiths, G., and Berg, T. (1996) J. Cell Sci. 109, 2905–2914
31. Griffiths, G., Hoflack, B., Simons, K., Mellman, I., and Kornfeld, S. (1988) Cell 52, 329–341
32. Chavrier, P., Parton, R. G., Hauri, H. P., Simons, K., and Zerial, M. (1990) Cell 62, 317–329
33. Schmid, S., Fuchs, R., Kielian, M., Helenius, A., and Mellman, I. (1989) J. Cell Biol. 108, 1291–1300
34. Wherrett, J. R., and Huterer, S. (1972) J. Biol. Chem. 247, 4114–4120
35. Brotherus, J., Renkonen, O., Fischer, W., and Herrmann, J. (1974) Chem. Phys. Lipids 13, 178–182
36. Huterer, S., and Wherrett, J. R. (1974) Can. J. Biochem. 52, 507-513
37. Bright, N. A., Reaves, B. J., Mullock, B. M., and Luzio, J. P. (1996) J. Cell Sci. 110, 2927–2940
38. Futter, C. E., Pearse, A., Hewlett, L. J., and Hopkins, C. R. (1996) J. Cell Biol. 132, 1011–1023
39. Mullock, B. M., Bright, N. A., Fearon, C. W., Gray, S. R., and Luzio, J. P. (1998) J. Cell Biol. 140, 591–601
40. Ohashi, M., Miwako, I., Nakamura, K., Yamamoto, A., Murata, M., Ohnishi, S., and Nagayama, K. (1999) J. Cell Sci. 112, 1125–1138
41. Andrejewski, N., Punnonen, E-L., Gudde, G., Tanaka, Y., Lullmann-Rauch, R., Hartmann, D., von Figura, K., and Saftig, P. (1999) J. Biol. Chem. 274, 12692–12701