Isolation and Characterization of Human Lysosomal Membrane Glycoproteins, h-lamp-1 and h-lamp-2

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Two major lysosomal membrane glycoproteins with apparent Mr ~ 120,000 were purified from chronic myelogenous leukemia cells. These glycoproteins are major glycoproteins containing polylactosaminoglycan and represent approximately 0.1–0.2% of total cell proteins. A monoclonal antibody specific to one of the glycoproteins and polyclonal antibodies specific to the other glycoprotein were obtained. Immunoelectron microscopic examination of HeLa cells revealed that these two glycoproteins mainly reside in lysosomes and multivesicular bodies. Immunoprecipitation experiments showed that a number of different cell lines express these glycoproteins. However, the apparent molecular weights differed between cell lines; this probably represents differences in the amount of polylactosaminoglycan expressed by each cell line. As shown in the following paper (Fukuda, M., Viitala, J., Matteson, J., and Carlsson, S. R. (1988) J. Biol. Chem. 263, 18920–18928) one of the glycoproteins is very homologous to that of a mouse counterpart, m-lamp-1. The human form of this glycoprotein is therefore named human lamp-1 (h-lamp-1), while the other glycoprotein, to which the monoclonal antibody was made, is called human lamp-2 (h-lamp-2).

Pulse-chase labeling experiments detected that h-lamp-1 and h-lamp-2 are produced first as precursor forms of 87.5 and 84 kDa, and treatment with endo-β-N-acetylglucosaminidase H (endo-H) or endo-β-N-acetylglucosaminidase F (endo-F) reduced their molecular masses to 39.5 and 41.5 kDa, respectively. It was estimated that h-lamp-1 has 18 N-linked saccharides and h-lamp-2 16, based on the results of partial digests with endo-F. These results indicate that the two lysosomal membrane glycoproteins are extensively modified by N-glycans, and some of these were found to have polylactosaminyl repeats and sialic acid. Human lamp-1 and lamp-2, therefore, serve as good models for understanding polylactosaminoglycan formation and the biosynthesis and processing of polylactosaminoglycan-containing glycoprotein.

Polylactosaminoglycans are heterogenous saccharides often having high molecular weights. They are distinguished from complex-type N-linked saccharides by having long side chains of Galβ1→GlcNAcβ1→3 repeats, which are susceptible to endo-β-galactosidase (for review see Ref. 1). Polylactosaminoglycans carry various antigenic structures such as ABO blood group antigens (2, 3), developmental antigens such as mouse F9 antigens (4) and human fetal (i) erythrocyte antigen (5), and tumor-associated antigens such as sialyl Le" (6). More recently, it has been shown that the lack of polylactosaminoglycan on the human erythrocyte anion transporter (Band 3) causes the glycoprotein to aggregate, resulting in abnormal membrane structures in a congenital dyserythropoietic anemia-type II (7, 8).

In human erythrocytes, polylactosaminoglycans are attached to Band 3 and Band 4.5 (which includes glucose transporter) (2, 3, 5), but not to glycoporphins (9, 10). On the other hand, little is known about the protein carriers for polylactosaminoglycan in nucleated cells. Since only a limited number of glycoproteins contain polylactosaminoglycan, as shown on PA-1 human teratocarcinoma cells (11), it is likely that the nature of a protein determines whether it is modified by polylactosaminoglycan.

We have shown previously that glycoproteins with Mr ~ 120,000 are major carriers for polylactosaminoglycan in granulocytic cells (12). In order to characterize the molecules which carry polylactosaminoglycan in nucleated cells, we isolated those glycoproteins. The data presented in this report indicate that these glycoproteins are lysosomal membrane glycoproteins, a group of proteins extensively glycosylated by N-linked saccharides.

EXPERIMENTAL PROCEDURES

RESULTS

Purification of Lysosomal Membrane Glycoproteins and Production of Specific Antibodies—We have previously shown that sialoglycoproteins with Mr ~ 120,000 in granulocytic cells contain significant amounts of polylactosaminoglycan (12). In order to isolate the glycoprotein(s), total cell lysates were applied to a column of wheat germ agglutinin-agarose, and the bound glycoproteins were eluted with 100 mM N-acetylglucosamine. The eluted glycoproteins were subjected to pre-

1 Portions of this paper (including "Experimental Procedures," Tables I and II, and Figs. 2, 4, 7, and 12) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
parative SDS\(^2\) gel electrophoresis. The material migrating between \(M_c \sim 100,000\) and \(130,000\) was eluted from gels and subjected to affinity chromatography with anti-leukosialin antibodies coupled to Sepharose. The glycoprotein fraction, which was not bound to the column, appeared to be homogenous when analyzed by SDS gel electrophoresis (Fig. 1, lane A). This purified glycoprotein fraction was initially used for immunization of a rabbit. The glycoproteins were isolated from the total lysate, but further analysis showed them to be lysosome-associated membrane proteins (lmp) with \(M_c \sim 120,000\). As shown below, this purified glycoprotein fraction contained two lysosomal membrane glycoproteins with similar molecular weights, and the antibodies produced were found to be specific to two lysosomal membrane glycoproteins, h-lamp-1 and h-lamp-2 (see also Ref. 29).

**Lysosomal Membrane Glycoproteins Contain Saccharides Susceptible to Endo-\(\beta\)-galactosidase**—The lysosomal membrane glycoprotein fraction was susceptible to endo-\(\beta\)-galactosidase treatment (Fig. 1, lanes A and B), indicating that the glycoprotein contains polylactosaminoglycan (1, 8). In contrast, leukosialin isolated from the same CML cells was barely affected by endo-\(\beta\)-galactosidase treatment (Fig. 1, lanes C and D). The mobility of the lysosomal membrane glycoproteins in SDS-polyacrylamide gels was not significantly altered after sialidase treatment (Fig. 2, lanes D and H), while leukosialin was found as a more slowly migrating band after sialidase treatment (Fig. 2, lanes A and B). This increase of apparent molecular weight is characteristic of glycoproteins containing a high amount of O-linked saccharides, as shown previously (14). These results suggest that lysosomal membrane glycoproteins and leukosialin differ significantly in glycosylation. Neither the lysosomal membrane glycoproteins nor leukosialin were affected greatly in mobility by reduction of disulfide bonds (Fig. 2, lanes E–H), indicating monomeric structures of the glycoproteins.

**Detection of Lysosomal Membrane Glycoproteins in Various Human Cell Lines**—A number of human cell lines were metabolically labeled with \([3H]\)glucosamine and subjected to immunoprecipitation with the lamp-specific antibodies. Immuno-precipitates were then analyzed by SDS gel electrophoresis before and after endo-\(\beta\)-galactosidase treatment. As shown in Fig. 3, all of the cell lines tested contain the lysosomal membrane glycoproteins and two additional points were noted: 1) the molecular weights of the glycoproteins differ significantly among different cell lines, and 2) the susceptibility to endo-\(\beta\)-galactosidase differs among cell lines tested. The latter characteristic appears to be directly correlated to the level of polylactosamine addition expressed by the different cell lines (see below). The glycoproteins were detected in all cells tested except erythrocytes.

**Detection of Precursors of Lysosomal Membrane Glycoproteins in HL-60 Cells**—In order to obtain information on the polypeptide and carbohydrate moieties of the lysosomal membrane glycoproteins, pulse-chase experiments were carried out. Fig. 4A shows that a precursor form of \(\sim 85\) kDa was detected immediately after the pulse labeling, and this precursor form was gradually converted to the mature form with a smeared, heterogeneously migrating band of \(M_c \sim 125,000\). The conversion of the precursor to mature forms was found to have \(t_c \sim 45\) min. An apparent heterogeneity in the time required for processing of the precursor form to the mature form was noticed, and a small amount of the precursor form was still detected after 120 min of chase. It was also noted that the precursor form appeared to consist of two closely spaced bands (Fig. 4A). In order to elucidate the manner in which the precursor and mature forms are glycosylated, the same pulse-labeled glycoproteins were digested with endo-H to remove N-linked carbohydrates. Fig. 4B shows that the precursor form was converted to two bands with apparent molecular weights of \(\sim 41,500\) and \(\sim 39,500\). As shown by its susceptibility to endo-H (compare at 120 min with or without endo-H), the mature form obtained after 120 min chase still contained a small number of high-mannose saccharides.

In order to estimate how many N-linked saccharides are present in the glycoproteins, the precursor form was digested with endo-F for increasing periods of time. From the results shown in Fig. 5, 19 bands were counted from the upper bands of the precursor to the lowest band after digestion, whereas 17 bands were counted from the lower band of the precursor to the second lowest band after digestion. These results, obtained on both HL-60 and K562 cells, suggest that the glycoproteins contain 16 to 18 N-linked saccharides, as further.

**The abbreviations used are:** SDS, sodium dodecyl sulfate; CML, chronic myelogenous leukemia; h-lamp-1 and h-lamp-2, human lysosomal-associated membrane protein-1 and protein-2; PMSF, phenylmethanesulfonyl fluoride; PBS, phosphate-buffered saline; endo-H, endo-\(\beta\)-N-acetylgalcosaminidase H; endo-F, endo-\(\beta\)-N-acetylgalcosaminidase F.

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![Fig. 1. Lysosomal membrane glycoproteins (lanes A and B) and leukosialin (lanes C and D) before and after endo-\(\beta\)-galactosidase treatment. The glycoprotein fraction containing human lamp-1 and lamp-2 and leukosialin were labeled with \(^{131}I\). The labeled glycoproteins were incubated with (lanes B and D) and without (lanes A and C) endo-\(\beta\)-galactosidase and subjected to SDS-polyacrylamide gel electrophoresis (7.5–12.5% acrylamide gradient). Gels were subjected to autoradiography.](image-url)
with unlabeled methionine for 10 min. Lysosomal membrane glycoprotein precursors were immunoprecipitated with anti-(h-lamp-1) and precipitated with anti-(h-lamp-2) antibody to react with only one of the precursors before endo-H digestion.

**Fig. 5.** Partial digestions with endo-F of lysosomal membrane glycoprotein precursor forms from HL-60 and K562 cells. Cells were labeled with [35S]methionine for 10 min and chased with unlabeled methionine for 10 min. Lysosomal membrane glycoprotein precursors were immunoprecipitated with anti-(h-lamp-1) and h-lamp-2, and were untreated (lane 1), or treated with endo-F for 5 min (lane 2), 20 min (lane 3), 45 min (lane 4) and 24 h (lane 5). Similar results were obtained when treated with endo-H. Open arrow shows unrelated coprecipitated radioactive material.

**Fig. 6.** Immunoprecipitation of human lamp-1 and lamp-2 and their precursors. HL-60 cells were labeled with [35S]methionine for 10 min, and the label was chased with unlabeled methionine for 10 min (B and C) or for 120 min (A). Cell lysates were immunoprecipitated with anti-(h-lamp-2) antibody (lanes 3) followed by anti-(h-lamp-1) antibodies (lanes 2). Lamp-1 and h-lamp-2 were coimmunoprecipitated with anti-(h-lamp-1 + h-lamp-2) antibodies (lanes 1). The glycoproteins were subjected to SDS gel electrophoresis after treatment with (C) or without (A and B) endo-H digestion.

The results also suggest that there is no difference in the number of N-glycans attached to lamps between HL-60 and K562 cells.

**Isolation of Two Lysosomal Membrane Glycoproteins from the Purified Glycoprotein Fraction**—The results shown in Figs. 4 and 5 suggested that the purified glycoprotein fraction actually consists of two different lysosomal membrane glycoproteins. In fact, the monoclonal antibody, obtained by immunizing with the purified glycoprotein fraction, was found to react with only one of the precursors before endo-H digestion in an immunoprecipitation assay (Fig. 6B, lane 3). We have designated the glycoprotein which reacts with the monoclonal antibody h-lamp-2, and the one which does not react, h-lamp-1. This nomination is based on the findings that human lamp-1 has an amino acid sequence homologous to lamp-1 isolated from mouse (see the following paper, Ref. 30). Before endo-H digestion, the precursor of h-lamp-2 migrated faster (lower M<sub>r</sub>) than that of h-lamp-1 (Fig. 6B). After endo-H digestion, however, h-lamp-2 migrated slower (greater M<sub>r</sub>) than h-lamp-1 (Fig. 6C). Based on the difference in molecular weights before and after endo-H digestion, it was deduced that human lamp-1 and lamp-2 contain 18 and 16 N-linked oligosaccharide chains, respectively.

**Isolation of h-lamp-1 and h-lamp-2 by Affinity Chromatography Employing a Monoclonal Antibody**—The anti-(h-lamp-2) monoclonal antibody was coupled to Sepharose, and the purified glycoprotein fraction, which contained human lamp-1 and lamp-2, was applied to the column. The glycoprotein, h-lamp-1, which was not bound, was pooled and purified further by Sephacryl S-300 gel filtration. The glycoprotein eluted from the column, h-lamp-2, was also subsequently purified by gel filtration. Each purified glycoprotein migrated as a single broad band when examined by SDS gel electrophoresis. The glycoproteins were detected more easily by periodate-Schiff reaction than by Coomassie Blue staining (Fig. 7). Furthermore, purified lamp-2 showed a higher apparent molecular weight than lamp-1. Each of the purified glycoproteins was subjected to Edman degradation for determining its NH<sub>2</sub>-terminal sequence, and both preparations showed a single amino acid in each step of Edman degradation, confirming that each glycoprotein was highly purified (Table I).

**Amino Acid and Carbohydrate Composition of Human lamp-1 and lamp-2**—Table 1 summarizes the characteristics of human lamp-1 and lamp-2. Both glycoproteins contain large amounts of carbohydrate which contribute to about 60% of each protein. The carbohydrate composition indicates that the glycoproteins contain mainly N-linked saccharides with possibly a small amount of O-linked saccharides, since N-acetylgalactosamine was detected. Both also contain a significant amount of sialic acid. Both lamp-1 and lamp-2 contain cysteine or cystine, while lamp-1 contains more methionine but less aspartic acid (+asparagine) than lamp-1 (Table II).

We believe that the glycoprotein fraction not bound by anti-leukosialin antibody (the material shown in Fig. 1A) consists of only lamp-1 and lamp-2 for the following reasons. First, the amino acid compositions of the starting material is clearly in agreement with the average of lamp-1 and lamp-2 (Table II). Second, the amino acid sequences obtained in the starting material gave 2 residues in each step, the same residues which were found in purified lamp-1 and lamp-2.

**Immunolocalization of Lysosomal Membrane Glycoproteins**—Application of the two rabbit antisera, anti-(lamp-1 + lamp-2) and anti-(lamp-1) to ultrathin sections from HeLa or MCF7 cells yielded the same pattern of staining. However, the anti-(lamp-1 + lamp-2) serum gave consistently more intense labeling over all positive structures. Specific immunolabeling was observed over lysosomes (as evidenced by cytochemically detectable acid phosphatase activity), greatly varying in size and shape, and found either adjacent to the trans side of the Golgi apparatus or in the remaining cytoplasm (Figs. 8, A and C, and 9, A and B). Gold particles were preferentially located at the luminal side of the lysosomal membrane as well as over amorphous material present in the lysosome lumen.

In parallel to these experiments, the Datura stramonium lectin was employed in a cytochemical affinity technique for the detection of N-acetyllactosaminyl residues in lysosomes. It has been shown that D. stramonium lectin binds to N-acetyllactosaminyl residues and poly-N-acetyllactosamine (25, 26). As shown in Fig. 8, B and D, such residues were detectable at the luminal side of the limiting membrane of variously sized and shaped lysosomes, as well as over the amorphous content material. In similar types of lysosomes...
particles, although irregularly shaped vesicular structures in lysosomes
lamp-2 immunoreactivity and N-acetyllactosaminyl residues with D).
the pattern of immunolabeling for lysosomal membrane glycoproteins corresponded strikingly to the distribution of N-acetyllactosaminyl residues. Anti-(h-lamp-1 + h-lamp-2) serum was used in A and C. Magnification: A, ×24,800; B, ×28,200; C, ×33,600; D, ×27,600.

In addition to lysosomes, other subcellular compartments showed positive labeling of gold particles. Multivesicular bodies exhibited specific labeling along their limiting membrane as well as at the level of the internal vesicles (Fig. 9C). Structures resembling peripheral endosomes were free of gold particles, although irregularly shaped vesicular structures closely located to them were labeled (Fig. 9, D and E). Plasma membrane labeling was only observed with anti-(lamp-1 + lamp-2) serum (Fig. 9B). This antiserum gave also weak immunolabeling over the trans side of the Golgi apparatus (Figs. 8C and 9F). No gold particles were detectable over the nucleus, mitochondria, or endoplasmic reticulum. When the antiserum was replaced by the preimmune serum, no gold particles were observed (not shown) confirming the specificity of the above described immunolabel.

H-lamp-2 Derived from Different Cell Lines Also Shows Different Molecular Weights—As shown above, the apparent molecular weight of mature h-lamp-2 is larger than h-lamp-1. The results shown in Fig. 3 could be, therefore, due to differences in the relative proportion of human lamp-1 and lamp-2 in different cells. In order to test this possibility, h-lamp-2 produced in various cells was immunoprecipitated by the monoclonal antibody. As shown in Fig. 10, h-lamp-2 molecules from various cell lines exhibit significantly different molecular weights. These results indicate that both lamp-1 and lamp-2 give different molecular weights depending on cell types.

Characterization of Carbohydrate Moiety of Human lamp-1 and lamp-2—In order to preliminarily characterize the carbohydrate moieties of human lamp-1 and lamp-2, glycoproteins were obtained by sequential immunoprecipitation of [3H]glucosamine-labeled K562 and HL-60 cells. After Pronase digestion and Sephadex G-50 gel filtration, glycopeptides obtained from lamp-1 of K562 cells showed essentially two peaks: the glycopeptides which eluted at the void volume (fractions 28-40) and those eluted at fractions 50-70 (Fig. 11A). The two glycopeptide fractions were separately subjected to mild alkaline degradation. The glycopeptides eluted at the void volume were converted to saccharides with low molecular weights after this treatment (Fig. 12A), indicating that these saccharides are O-linked oligosaccharides. When the O-linked oligosaccharides were applied to a Bio-Gel P-4 column, they eluted at the positions consistent with NeuNAca2→3Galβ1→3(NeuNAca2→6)GalNAcOH and NeuNacβ2→6(Galβ1→3)GalNAcOH (data not shown). In contrast, the glycopeptides which eluted in fractions 50-70 of
the Sephadex G-50 column were not changed after the same peptide treatment (data not shown). When the O-linked saccharides from HL-60 lamp-2 were subjected to Bio-Gel P-4 gel filtration, they eluted at the positions corresponding to NeuNAcα2→3Galβ1→4GlcNAcβ1→6 NeuNAcα2→3GalNAcOH and Galβ1→4GlcNAcβ1→6 NeuNAcα2→3GalNAcOH (Fig. 12F). Finally, the glycopeptides b eluted later than IgG glycopeptides, and they were susceptible to endo-H to yield high-mannose oligosaccharides which eluted between fractions 66 and 84 (Fig. 12C). These results indicate glycopeptides b are high-mannose saccharides.

The glycopeptides obtained from HL-60 lamp-1 showed an intermediate elution profile between K562 lamp-2 and HL-60 lamp-2 glycopeptides. The N-glycans of HL-60 lamp-1 (a of Fig. 11C) are not as large as those of HL-60 lamp-2 and were only partially digested by endo-β-galactosidase (Fig. 11E). These results suggested that both lamp-1 and lamp-2 of HL-60 cells contain much more polylactosaminoglycan than those of K562 cells, and lamp-2 contains more polylactosaminoglycan than lamp-1 in HL-60 cells. The latter data explain why mature lamp-2 is larger than mature lamp-1, even though the precursor of lamp-2 is smaller than that of lamp-1. These results also indicate that human lamp-1 and lamp-2 contain O-linked saccharides and high-mannose saccharides in addition to sialylated N-linked saccharides, some of which are polylactosaminoglycans.

**DISCUSSION**

The present report describes the isolation, characterization, and distribution of two human lysosomal membrane glycoproteins, h-lamp-1 and h-lamp-2. These two glycoproteins have polypeptide portions of about 40-kDa and are heavily glycosylated by N-glycans; h-lamp-1 and h-lamp-2 contain 18 and 16 N-glycans, respectively. Most strikingly, some of the N-glycans are polylactosaminoglycans. It is interesting to note that the pattern of distribution for lamps and D. stramonium lectin binding sites is strikingly similar (Fig. 8). In both cases, a spot-like labeling is often seen along the luminal side of the lysosomal membrane. These results are consistent with the findings that D. stramonium lectin preferentially binds to poly-N-acetyllactosaminyl structures (25). Thus, our results obtained by cytochemistry and characterization of saccharides attached to h-lamp-1 and h-lamp-2 agree well and point toward the fact that human lamp-1 and lamp-2 are major sialoglycoproteins carrying polylactosaminoglycan. Similar glycoproteins have previously been detected by monoclonal antibodies in mouse (31, 32), rat (33, 34), and chicken (35, 36) cells. However, the previous studies did not characterize lysosomal membrane glycoproteins with Mr ~ 120,000 as carriers for polylactosaminoglycan.

It is noteworthy that human lamp-1 and lamp-2 exhibit significantly different molecular weights depending on cell types (Figs. 3 and 10). For example, lambs from HL-60 cells showed much higher molecular weights than those from K562 cells. As shown in Fig. 5, however, the lamps contain the same number of N-glycans regardless of cell types. The results shown in Figs. 11 and 12 demonstrate that lamps from HL-60 cells contain a significant amount of polylactosaminoglycan, whereas lamps from K562 cells essentially lack polylactosaminoglycan. These combined results indicate that the variation in molecular weight is most likely due to differential processing, particularly in the attachment of polylactosaminoglycan. The present results on the content of polylactos-
aminoglycan in lamp-1 and lamp-2 are also consistent with those reported previously on total cellular glycopeptides. It has been shown that K562 cells have minimal amounts of polylactosaminoglycan (37), whereas granulocytic cells (38), HL-60 cells (39), and PA-1 cells (11) express significant amounts of polylactosaminoglycan. Very recently, we have succeeded in isolating cDNAs for human lamp-1 (29, 30) and lamp-2 (30) and the sequences of those cDNAs are consistent with the conclusion that human lamp-1 and lamp-2 contain, respectively, 18 and 16 N-glycosylation sites. Furthermore, the attachment of polylactosamine at some of the glycosylation sites, make the carbohydrate moieties relatively bulky. Since the major portions of the molecules presumably reside in the luminal side of lysosomes, this large carbohydrate moiety probably serves to protect lysosomal membrane glycoproteins from degradation by lysosomal proteases. Experiments have also shown that h-lamp-1 and h-lamp-2 indeed are unusually resistant to a variety of different proteases (data not shown).

The isolated glycoproteins contain a significant amount of polylactosaminoglycan and sialic acid, suggesting that the glycoprotein traverses the trans-Golgi cisternae (40). It is unlikely that the carbohydrate moiety is serving as a marker for targeting the molecules to lysosome, since tunicamycin treatment does not inhibit the transport of the glycoproteins to lysosomes (34). In this context, it is interesting to compare the present results with those obtained on mannose-6-phosphate receptors. As shown previously (41, 42), the receptor-lysosomal enzyme complex exits from the Golgi apparatus, most likely from the trans-Golgi. Lysosomal enzymes have been shown to contain mannose-6-phosphate modification of carbohydrates, and a specific receptor for this structure serves to route the enzymes toward the lysosome (43, 44). The receptor-enzyme complex is transported to the prelysosomal compartment where the enzymes are dissociated from the receptors (41, 42). The receptors then recycle to the Golgi apparatus and repeat the process, and thus, the mannose-6-phosphate receptors do not reach lysosomes. On the other hand, lysosomal membrane glycoproteins are mainly present in lysosomes and much less are found in other compartments. These results suggest that a molecular signal for targeting of lysosomal membrane glycoproteins to lysosomes is different from that for mannose-6-phosphate receptor routing. In fact, no similarity exists in amino acid sequences between lysosomal membrane glycoproteins (see the following paper (30)) and mannose-6-phosphate receptors (45-48). Lysosomal membrane glycoproteins can also be detected in plasma membranes, multivesicular bodies, and possibly the trans-Golgi (Figs. 8 and 9; see also Refs. 32, 36, and 49). It is possible that lysosomal membrane glycoproteins are somehow involved in the dynamics of lysosomes such as in the process of fusion of lysosomes with various other organelles.

It is interesting to compare some of the properties of human lamp-1 and lamp-2 presented here to those reported by others. For example, Lewis et al. (33) showed the presence of 19 N-glycosylation sites in rat lysosomal membrane glycoprotein(s) Lgp 120 by endo-H digestion. They detected two protein bands on SDS-polyacrylamide gel electrophoresis after 20 h of digestion, and the ratio of these bands is approximately the same as seen here in Fig. 5, which represents lamp-1 and lamp-2. On ~120-kDa glycoproteins of chicken and mouse cells, Lipincott-Schwartz and Fambrough (35) and Green et al. (40) showed a similar half-time for maturation of about 50 min and a similar doublet peptide after endo-H treatment. As shown in the following paper (30), the amino acid sequences of lamp-1 from human, mouse, and chicken are more than 50% identical. These results clearly indicate that lysosomal membrane glycoproteins in these different species are closely related to each other.

Our studies show that human lamp-1 and lamp-2 are major glycoproteins of the total cell mass. Although we did not attempt to isolate glycoproteins specifically from lysosomes, the glycoproteins obtained were derived from lysosomes. Similarly, Hughes and August (32) isolated mouse lamp-1 from total membranes. Additionally, lamp-1 and lamp-2 are major polylactosaminoglycan-carrying glycoproteins in various cells, and we have found them each to contain a significant amount of polylactosaminoglycan (Figs. 3, 8, and 11). These results led us to reevaluate some of the previous reports on polylactosaminoglycan-containing glycoproteins. For example, Dennis et al. (50) reported that metastatic cells contain more GlcNAcβ1→6 branching on α-mannose than nonmetastatic cells. Since this branching is the most preferable site for polylactosamine extension (25, 51, 52), more polylactosamine is present in metastatic cells than nonmetastatic cells as a result. These structures mainly reside on Gp 130 in mouse cells and it is likely that Gp 130 is the mouse counterpart of lamp-1 or lamp-2 since both Gp 130 and lamps represent a major carrier of polylactosaminoglycan. It has been reported from several laboratories that tumor cells invade surrounding tissue or penetrate the endothelial membrane by secreting lysosomal enzymes (53-55). It will be intriguing to know how this increased secretion takes place in tumor cells and if any correlation exists between the secretion of lysosomal enzymes and surface expression of lysosomal membrane glycoproteins.

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REFERENCES

EXPERIMENTAL PROCEDURES

Sucrose gradient centrifugation. Lysosomal membranes were centrifuged in a Beckman SW 39 rotor. The packed CML cells were homogenized in 0.25 M sucrose, 150 mM KCl, 10 mM Tris-HCl, pH 7.4. After clarification on tea for 30 min, the supernatant was centrifuged for 2 h at 100,000g, and the pellet was collected.

Glycoproteins were isolated by the high-speed centrifugation through the columns of 8% sucrose in 10 mM Tris-HCl, pH 7.4, 0.2% EDTA, 100 mM NaCl, 50 mM NP-40, 1% diethylamine, pH 12. Eluted fractions were dialyzed against 61 mM Tris-HCl, pH 7.4. After elution on tea for 30 min, the supernatant was centrifuged for 2 h at 100,000g, in the ultracentrifuge was collected.

During the performance of column chromatography, the gel was washed with 50 ml of 50 mM sucrose, 0.1 M NaCl, 50 mM Tris-HCl, pH 8.0, and with 20 ml of 1 M sucrose, 0.1 M NaCl, 50 mM Tris-HCl, pH 8.0. Eluate was putted in a column of 0.3 ml 50% sucrose, 0.1 M NaCl, 50 mM Tris-HCl, pH 8.0, with a flow rate of 3 ml/h. Fractions of 0.6 ml were collected and assayed for 125I-

The obtained material was collected to contain small amounts of unknown, which was removed by passing twice through a column of 50 mg column of 50 mg column 2000 ml of 1 M sucrose, 0.1 M NaCl, 50 mM Tris-HCl, pH 8.5. After elution on tea for 30 min, the supernatant was centrifuged for 2 h at 100,000g, in the ultracentrifuge was collected.

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The obtained material was found to contain small amounts of unknown, which was removed by passing twice through a column of 50 mg column of 50 mg column 2000 ml of 1 M sucrose, 0.1 M NaCl, 50 mM Tris-HCl, pH 8.5. After elution on tea for 30 min, the supernatant was centrifuged for 2 h at 100,000g, in the ultracentrifuge was collected.
Poly lactosaminoglycan-containing Lysosomal Membrane Glycoproteins

Immunohistochemistry - Ultrathin sections from Lowicryl HM embedded cells were processed with the protein A-gold technique (24). In short, grids were floated on a drop of 0.5% ovalbumin in PBS for 5 min and then transferred to droplets (10 μl) of anti-(h-lamp-1 + h-lamp-2) serum (20-fold dilution or anti-(h-lamp-1 + h-lamp-2) serum (20-fold dilution) either for 2 h at room temperature or overnight at 4°C. This was followed by two rinses for 2 min each with PBS and incubation with protein A-gold complex (1 nm gold particles, diluted with PBS containing 1% bovine serum albumin, 0.075% Triton X-100 and 0.075% Tween 20 to an absorbance of 0.00 at 525 nm) for 1 h at room temperature. Sections were then washed in water and air dried before staining with uranyl acetate. In control immunostains, the sera were replaced with preimmune serum (20–25 fold dilution).

Leucin Carbohydrate - The Galactosia. lectin was used for the detection of N-acetyl-lactosaminyl residues (25, 26) in the postcytolythic fraction. Briefly, ultrathin sections of Lowicryl HM embedded Hela cells were incubated for 1 h with 0.5 μg/ml of lectin in PBS and incubation with commercial-gold complex (1.0 μg/ml, 0.25, 30 μm). Specificity of lectin staining was controlled by preincubation of the lectin with 0.5 μg/ml of N-acetyllactosamine, and by pretreatment of the thin sections with endo H (27).

Acid Phosphatase Carbohydrate - Activity for acid phosphatase was demonstrated with cytidine 5'-monophosphate as substrate as described previously (28).

FIG. 2
Effect of reduction and treatment with neuraminidase.

FIG. 4
Pulse-chase experiment with HL-60 cells.

A. Cells were labeled with [35S]methionine for 10 min. and chased the radioactivity with unlabeled methionine for indicated periods of time. The lysosomal membrane glycoproteins were immunoprecipitated by anti-(h-lamp-1 + h-lamp-2) antibodies and analyzed by SDS-polyacrylamide gel electrophoresis (7% acrylamide) under non-reducing (A-D) or reducing (E-H) conditions.

FIG. 7
Analysis of purified human lamp-1 and lamp-2 by SDS-polyacrylamide gel analysis.

Lanes 1 are molecular weight standards (1 μg each). Five μg of proteins, corresponding to approximately 1 μg of h-lamp-1 or h-lamp-2 was analyzed on a 7% acrylamide gel. Lane 2, h-lamp-1; Lane 3, h-lamp-2. Cells were obtained after Comassie Blue staining (A) or periodate-Schiff staining (B).

FIG. 12
Gel filtration of sialoglycopeptides obtained from human lamp-1 and lamp-2.

A. Sephadex G-50 gel filtration of alkaline borohydride-treated glycopeptides with high molecular weights (fractions 28–40 in Fig. 11A). Similar profiles were obtained from other glycopeptides with high molecular weights (fractions 28–40 in Fig. 11A, C and D).

B. Bio-Gel P-4 gel filtration of fractions 64–80 in Fig. 12A. The 6 and 5 denote the elution position of Neu5Ac2-3Galβ1-3GlcNacβ1-4GlcCer (Neu5Ac2-3Galβ1-3GlcNacβ1-4GlcCer, Neu5Ac2-3Galβ1-3GlcNacβ1-4GlcCer and Neu5Ac2-3Galβ1-3GlcNacβ1-4GlcCer).

C. Sephadex G-50 gel filtration of glycopeptide B in Fig. 11D after endo-A-N-acetylglucosaminidase VI treatment. Manα and Manβ denote the elution positions of ManαGlcNAc2 and ManβGlcNAc2.
<table>
<thead>
<tr>
<th></th>
<th>LAMP-1</th>
<th>LAMP-2</th>
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<tbody>
<tr>
<td>Mr of mature glycoprotein</td>
<td>129K</td>
<td>125K</td>
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<tr>
<td>Mr of precursor glycoprotein</td>
<td>87.5K</td>
<td>94K</td>
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<tr>
<td>Mr of endo-H digested precursor</td>
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<td>41.5K</td>
</tr>
<tr>
<td>N-terminal sequence</td>
<td>ASN/FF/II/ SER</td>
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<tr>
<td>Asparagine residues</td>
<td>112</td>
<td>116</td>
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<tr>
<td>Glutamine residues</td>
<td>95</td>
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<tr>
<td>Alanine residues</td>
<td>35.4</td>
<td>38.2</td>
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<tr>
<td>Threonine residues</td>
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<tr>
<td>Valine residues</td>
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<td>55%</td>
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**Carbohydrate Composition, moles/mole**

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<thead>
<tr>
<th>GlcNAc</th>
<th>Fucose</th>
<th>Mannose</th>
<th>Galactose</th>
<th>GlcA</th>
<th>GalA</th>
<th>Sialic acid</th>
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<tr>
<td>98.9</td>
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<td>70.7</td>
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**Percent carbohydrate**

<table>
<thead>
<tr>
<th>LAMP-1</th>
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<td>52%</td>
<td>55%</td>
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**Amino Acid Composition of Human LAMP-1 and LAMP-2**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>LAMP-1</th>
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<tbody>
<tr>
<td>Asp</td>
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<td>12.7</td>
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<tr>
<td>Thr</td>
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<tr>
<td>Ser</td>
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<tr>
<td>Pro</td>
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<td>7.8</td>
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<tr>
<td>Arg</td>
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<td>8.2</td>
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<tr>
<td>Lys</td>
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<td>2.1</td>
</tr>
<tr>
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<tr>
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<tr>
<td>Arg</td>
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<td>5.1</td>
</tr>
<tr>
<td>Trp</td>
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<td>-0.3</td>
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</tbody>
</table>

a The amino acid composition of the material shown in Fig. 1a.
b The amino acid composition based on the amino acid sequences deduced from cDNAs (the following paper, ref. 36).
c Determined as 5-carboxymethyl cysteine.
d Tryptophan was not analyzed.