Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 7577–7581, October 1988
Cell Biology

Derived protein sequence, oligosaccharides, and membrane insertion of the 120-kDa lysosomal membrane glycoprotein (lgp120): Identification of a highly conserved family of lysosomal membrane glycoproteins

CHRISTINE L. HOWE*, BRUCE L. GRANGER*, MICHAEL HULL*, SAMUEL A. GREEN*, CHRISTOPHER A. GABEL†, ARI HELENIUS*, AND IRA MELLMAN*‡

*Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510; †Department of Anatomy and Cell Biology, Columbia University College of Physicians and Surgeons, New York, NY 10032

Communicated by Marilyn Gist Farquhar, June 13, 1988

ABSTRACT **The 120-kDa lysosomal membrane glycoprotein (lgp120) is an acidic, heavily glycosylated membrane protein enriched in the lysosomal membrane. To determine the basis for its selective transport to and stability in lysosomes, we have investigated the structure of lgp120. By using an oligonucleotide probe corresponding to the amino terminus of rat lgp120, we isolated and characterized cDNA clones containing the entire coding region. The deduced amino acid sequence demonstrates that lgp120 contains a putative signal peptide, 18 sites for N-linked glycosylation, a single membrane-spanning segment, and a short (11 amino acid) cytosolic tail. The sequence suggests a distinct domain organization, with two luminal glycosylated regions separated by a nonglycosylated proline-rich region. Proteolysis in detergent showed that the protein was not intrinsically resistant to exogenous or endogenous proteases. The N-linked oligosaccharides on lgp120, tetraantennary structures with two lactosamine repeats on one of the branches, were not different from those of glycoproteins on the plasma membrane. lgp120 was similar in its domain organization and portions of its amino acid sequence to the avian 100-kDa lysosomal membrane protein LEP100 [Fambrough, D. M., Takeyasu, K., Lippincott-Schwartz, J., Siegel, N. R. & Somerville, D. (1988) *J. Cell Biol.* 106, 61–67], and to a distinct 110-kDa lysosomal membrane protein (lgp110) that colocalizes with lgp120. The similarities between lysosomal membrane glycoproteins from diverse species, coupled with the fact that at least two distinct lysosomal membrane glycoproteins are expressed in a single species, indicate the existence of a conserved family of glycoproteins enriched in the lysosomal membrane.**

The lysosomal membrane has several important functions: it provides a stable container for acid hydrolases, it generates and maintains the acidic internal pH characteristic of lysosomes, it transports the products of hydrolysis out of the lysosomal lumen, and it specifically recognizes and fuses with a restricted subset of other cytoplasmic organelles.

A number of lysosomal membrane glycoproteins (lgps) that may be involved in these functions have been identified as being enriched in the lysosomal membrane (refs. 1–7; S. Schmid, H. Plutner, and I.M., unpublished data). Most of these have relatively small core polypeptides (20–60 kDa) that are heavily N-glycosylated and extremely anionic (pI 2–4) due to multiple sialic acid residues. The 120-kDa lgp (lgp120), for example, is an integral membrane protein (42-kDa polypeptide core) with 18 N-linked oligosaccharides and a pI value of <4 (1).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

The structural features of lgps that are responsible for their selective transport to lysosomes and their ability to survive in the hydrolase-rich environment are unknown. After leaving the Golgi, lgps are delivered rapidly to lysosomes ($t_{1/2}$ < 1 hr) yet they exhibit relatively long half-lives (10–20 hr) after arrival (8). The high concentration of N-linked oligosaccharide chains may play a role in the hydrolase resistance (9, 10), but it is not known whether the core polypeptide itself, either independently or in conjunction with the oligosaccharides, is somehow intrinsically resistant to degradation. Higher-order assembly or interaction of lgps with each other or with other lysosomal components could also be involved.

To better characterize the structural features and functions of the lgps, we have isolated and sequenced a cDNA clone coding for rat lgp120§ and determined the glycoprotein's oligosaccharide structure. In conjunction with the sequence data for the avian 100-kDa lgp LEP100 (11) and the 110-kDa lgp (lgp110) (B.L.G., A.H., and I.M., unpublished data), our results suggest a high degree of conservation in the domain organization and primary structure of lgps and provide evidence for the existence of a family of gene products.

MATERIALS AND METHODS

Antibodies. Mouse anti-rat lgp120 monoclonal antibody Ly1C6 and polyclonal rabbit antisera prepared against affinity-purified rat lgp120 were made and used as described (1). Rat anti-mouse LAMP-1 (3) was generously provided by Pat D'Souza and Tom August (Johns Hopkins University).

Design of Oligonucleotides for cDNA Library Screening. lgp120 was purified from NRK cells by antibody-affinity chromatography essentially as described (1). A consensus amino acid sequence for the first 14 positions of lgp120 was derived from amino-terminal amino acid sequences of two preparations. Codons for each amino acid were chosen according to the optimal codon usage table of Lathe (12). A 41-base oligonucleotide was used to screen several λgt11 cDNA libraries by standard methods (13). Positive phage were identified in a rat liver cDNA library (RL1001b, Clontech, Palo Alto, CA), and the inserts were subcloned into M13mp19 (14) or Bluescript KS+ (Stratagene Cloning Systems, La Jolla, CA) vectors for sequencing. An RNA transcript of a 1.12-kilobase (kb) insert in Bluescript was used to screen two additional λgt11 cDNA libraries, a randomly primed neonatal rat brain library (RAP1) obtained from

Abbreviations: endo H, endoglycosidase H; FcR, Fc receptor; GlcNAc, *N*-acetylglucosamine; lgp, lysosomal membrane glycoprotein; lgp120, 120-kDa lysosomal glycoprotein.

‡To whom reprint requests should be addressed.

§The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03672).

Norman Davidson (Caltech) and a poly(dT)-primed rat heart library (RH1006, Clontech). Processing conditions were identical to those described (13), except that hybridization was done at 42°C and washes were at 65°C.

DNA Sequencing. Single-stranded DNA was isolated and sequenced by the dideoxy method (15) with either the Klenow fragment of DNA polymerase or modified T7 DNA polymerase (16) in the Sequenase system (United States Biochemical, Cleveland), and deoxyadenosine 5′-[α-[^{35}S]-thio]triphosphate (>600 Ci/mmol; 1 Ci = 37 GBq; Amersham). Both strands of the coding region of the cloned cDNA were sequenced, with sequence alignment and analysis accomplished with software from the Genetics Computer Group, University of Wisconsin (17).

Microsome Isolation and Protease Digestion. Microsomes were isolated from J774 cells pulse-labeled with [^{35}S]methionine for 10 min at 37°C and used for protease-protection experiments as described (18). To disrupt microsomal membranes, 0.1% Nonidet P-40 and 0.2% $NaDodSO_4$ were mixed with some aliquots in the presence or absence of bovine serum albumin at 10 mg/ml prior to addition of proteinase K. The membranes were lysed and immunoprecipitated with the rabbit anti-rat lgp120 polyclonal antibody (1, 18), omitting the Triton X-114 phase-separation step. After immunoprecipitation, samples were digested with endo-β-*N*-acetylglucosaminidase H (endo H) as described (18). Electrophoresis and fluorography techniques have been described (8).

Oligosaccharide Structure Analysis. J774 cells were incubated for 20 hr with [^{3}H]glucosamine (0.083 mCi/ml; 40 mCi/mmol; Amersham) to radiolabel all saccharides except mannose, and lgp120 and Fc receptor (FcR) glycoproteins were isolated by immunoprecipitation from cell lysates as described (1, 18). Immunoprecipitated proteins were resolved by $NaDodSO_4$/PAGE, and protein bands were excised from dried gels. These isolated proteins were digested with Pronase, and the resulting glycopeptides were subjected to further analysis by column chromatography and glycosidase digestions as described (19–21). Anti-LAMP-1 antiserum was used in these experiments because of its higher efficiency in immunoprecipitation; immunoprecipitations of detergent lysates of [^{35}S]methionine-labeled J774 cells with the polyclonal rabbit anti-rat lgp120 serum and a rabbit anti-LAMP-1 serum demonstrated the same mobilities by $NaDodSO_4$/PAGE for the mature antigens, the endo-H-sensitive precursors, and the forms synthesized in the presence of tunicamycin.

Nucleic Acid Hybridizations. RNA gel blot analysis of rat liver RNA was performed by standard technique (13) by using as a probe an RNA transcript of a 1.12-kb insert cloned into Bluescript. Hybridization conditions with this probe were identical to those used in screening.

RESULTS

Isolation of cDNA Clones Encoding Rat lgp120. After unsuccessful attempts to clone lgp120 by screening λgt11 libraries (22) with antibodies, we generated an oligonucleotide to use as a probe. Two independent determinations of the amino-terminal amino acid sequence of lgp120 isolated from rat NRK cells yielded the sequences (Met)-Pro-Ala-Leu-Xaa-(Gln)-Val, and Xaa-Xaa-Xaa-Leu-Phe-Xaa-Val-Lys-Asp-Asn-(His)-Gly-Thr-Ala-Xaa-Ile-Met; where residues in parentheses are tentatively identified. The data were combined to generate a consensus amino acid sequence Met-Pro-Ala-Leu-Phe-Gln-Val-Lys-Asp-Asn-His-Gly-Thr-Ala, which in turn was used to develop the sequence of a 41-base oligonucleotide (Fig. 1).

After screening cDNA libraries with the oligonucleotide probe or with an RNA transcript of a cDNA clone isolated with the oligonucleotide, we obtained clones extending for

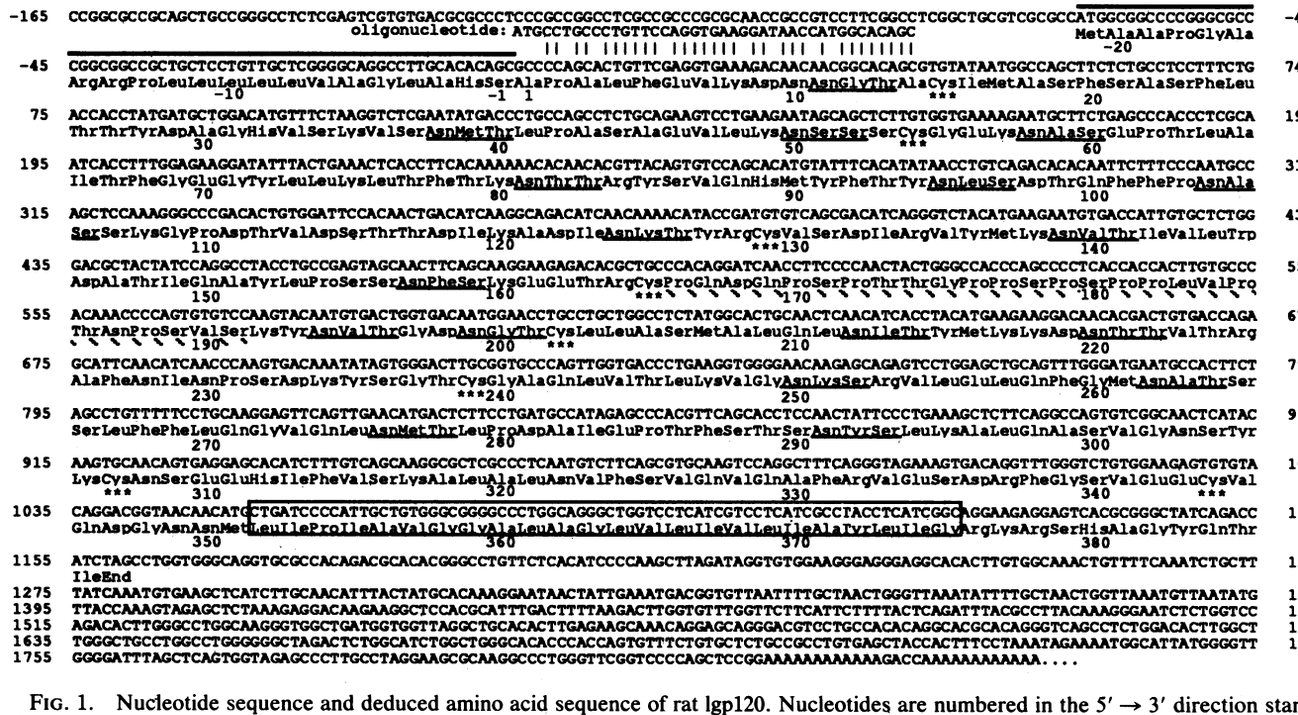

FIG. 1. Nucleotide sequence and deduced amino acid sequence of rat lgp120. Nucleotides are numbered in the 5′ → 3′ direction starting with the first nucleotide in the codon of the amino-terminal residue of the mature protein as zero; nucleotides on the 5′ side of this point have negative numbers and include residues encoding a putative signal peptide (indicated by a dark overline) and a 5′ untranslated region. Amino acids are numbered starting with the first residue in the amino terminus of the mature protein as 1; residues encoding a putative signal peptide have negative numbers. Above the nucleotides coding for amino acids 1–14 is the sequence of the 41-base synthetic oligonucleotide used to screen the cDNA libraries, with lines indicating exact matches with the lgp120 cDNA clone. The 18 sites for asparagine-linked glycosylation are underlined, and the eight cysteine residues are starred. The single potential membrane-spanning domain is indicated by a box, and the proline-rich domain is marked by diagonal underlines.

≈2.2 kb. RNA gel blot analysis of rat liver poly(A)$^+$ RNA indicates that the mRNA for lgp120 is a single species 2.7 kb long (data not shown). Thus, although the cDNAs contain the entire coding region, they lack ≈0.5 kb of sequence present in the mRNA.

The nucleotide sequence and deduced amino acid sequence of lgp120 are shown in Fig. 1. Starting at the amino terminus of the mature protein as determined by protein sequencing, lgp120 consists of 386 amino acids and has a predicted molecular mass of 41.8 kDa. The deduced amino-terminal sequence matches that obtained by amino acid sequencing, disregarding the amino acids whose assignment was uncertain (residues 1, 6, and 11).

As expected for an integral membrane protein, lgp120 has a presumptive amino-terminal signal peptide with typical features. This 21-amino acid sequence begins with the first methionine upstream from the amino terminus of the mature protein, and includes 2 basic residues near its amino terminus, a central hydrophobic region, and amino acids at positions −1 and −3 that correspond to a predicted signal cleavage site (23).

The mature lgp120 sequence is composed of an ectodomain followed by a single transmembrane region and short cytosolic tail. In the ectodomain are 18 canonical N-linked glycosylation sites, corresponding to the biochemical determination of the number of oligosaccharide chains in both rat and mouse lgp120 (1). All of these glycosylation sites are present in two domains of 10 and 8 sites that are separated by a stretch of 26 amino acids rich in proline, serine, and threonine. There are eight cysteines in lgp120, all in the ectodomain and arranged with a regular spacing of 35–38 residues (spaces of 38, 35, 36, and 36 residues) between adjacent pairs. Spacing between these pairs is variable (74, 36, and 67 amino acid residues). $NaDodSO_4$/PAGE performed in the presence and absence of reducing agents suggests that disulfide bonds may be present in lgp120 (electrophoretic mobility is slightly increased after reduction), but interchain disulfide bridges have not been detected (unpublished results).

A hydrophobicity plot (24) indicates (data not shown) a potential membrane-spanning domain of 24 amino acids near the carboxyl-terminal end of the sequence, bounded on the carboxyl side by three positively charged amino acids. Assuming that the oligosaccharides on lgp120 are in the lumen of the lysosome, this leaves a deduced cytosolic domain of 11 amino acids. There are three potential phosphorylation sites in this domain, but we have not been able to detect phosphate in lgp120 (ref. 1 and unpublished results). The amino acid sequence predicted from this clone thus matches lgp120 in size, amino-terminal sequence, number of glycosylation sites, presence of a presumed signal sequence, and one potential membrane-spanning domain.

Membrane Insertion and Protease Sensitivity of lgp120. To confirm the predicted orientation of lgp120 in the membrane and to determine whether its structure could account for the ability of lgp120 to withstand lysosomal proteolysis, we investigated the protease sensitivity of newly synthesized lgp120 inserted into the rough endoplasmic reticulum. Total microsomes were isolated from pulse-labeled J774 cells, a mouse macrophage cell line that is a particularly good source of lgp120 (8); cDNA cloning of murine lgp120 shows also that it is nearly identical to its rat homolog (>90% similar) (unpublished data). The susceptibility of [^{35}S]methionine-labeled lgp120 to proteinase K added in the presence and absence of detergent was determined by immunoprecipitation. Protease digestion of intact microsomes produced a barely detectable shift in the electrophoretic mobility of the 90-kDa lgp120 precursor, in accord with the small size of the cytosolic domain predicted from the cDNA sequence. Similar results were obtained if the lgp120 precursor was digested with endo H prior to $NaDodSO_4$/PAGE to better resolve the mobilities of the polypeptides themselves. Given a cytosolic tail of only 11 amino acids, a shift of no more than 1–1.5 kDa would be expected assuming complete digestion with proteinase K.

While almost entirely protected from proteolysis in intact microsomes, solubilization of the microsomes with detergent rendered lgp120 extremely sensitive to degradation (data not shown). Similar results were noted (8) when we observed that efficient precipitation of mature lgp120 from purified lysosomes was possible only when detergent lysis was carried out in the presence of a large excess of unlabeled protein. Thus, its stability to lysosomal proteases is unlikely to be due to an intrinsic resistance of its amino acid sequence to proteolysis.

Composition and Structure of lgp120 Oligosaccharide Chains. Given the large amount of carbohydrate, the stability or function of lgp120 might be due to the type or quantity of its oligosaccharides. To determine if the oligosaccharides of lgp120 were in some way unique, we analyzed their structure in comparison with those associated with the Fc receptor (FcR), a surface glycoprotein on J774 cells (18). Con A-Sepharose chromatography of [^{3}H]glucosamine-labeled glycopeptides isolated from both proteins showed the presence of tri- and/or tetraantennary N-linked or O-linked oligosaccharides (19), with <25% biantennary or high-mannose-type chains (Fig. 2). All of the ^{3}H-labeled lgp120 glycopeptides that did not bind to Con A-Sepharose were excluded during Bio-Gel P-10 chromatography, and mild alkaline hydrolysis did not alter the elution profile (data not shown); thus, the lgp120 glycopeptides were likely to contain N-linked rather than O-linked structures (25). After strong acid hydrolysis to monosaccharides (conditions that destroy sialic acid residues), only [^{3}H]glucosamine was detected by paper chromatography.

The structure of the [^{3}H]glucosamine oligosaccharides was examined in greater detail by glycosidase digestion. Neuraminidase released ≈10% of the radiolabel from both lgp120 and FcR-derived glycopeptides. Digestion with β-*N*-acetylglucosaminidase alone, or in combination with β-galactosidase,released only 0.9% and 16% of the [^{3}H]glucosamine-derived radioactivity, respectively. Combined treatment with all three enzymes, however, released 76% of the radiolabel as low molecular weight components. Thus, the majority of lgp120 oligosaccharides are sialylated. Assuming that the radioactivity resistant to the trio of exoglycosidases (24%) represents the two core *N*-acetylglucosamine (GlcNAc) residues found on all N-linked chains (26), then one residue of GlcNAc is equivalent to 12% of the radioactivity. Thus, the released radiolabel (excluding the 10% released as sialic acid) represents approximately five additional GlcNAc residues. Since tri- and tetraantennary chains are expected to contain only three or four GlcNAc residues, it is likely that the lgp120 oligosaccharides contain two repeating disaccharides (GlcNAc-Gal) in a polylactosamine-like structure (20). This fact was determined directly by digestion of the Con A-flow-through peptides with endo-β-galactosidase, and separation of the reaction products by Bio-Gel P-6 chromatography (Fig. 2, *B–F*).

In summary, the data suggest that the major N-linked oligosaccharides attached to lgp120 are tetraantennary structures in which one of the four branches (on average) is not sialylated, and one branch possesses a dilactosamine sequence. This structure does not appear to be distinct from that attached to a plasma membrane glycoprotein, the FcR.

Evolutionary Conservation of the lgp120 Domain Structure. Examination of the predicted amino acid sequence for lgp120 suggests that the mature protein is composed of five distinct domains: two glycosylated domains of the luminal region separated by a proline-rich domain, a membrane spanning region, and an 11-amino acid cytosolic tail. One of the striking

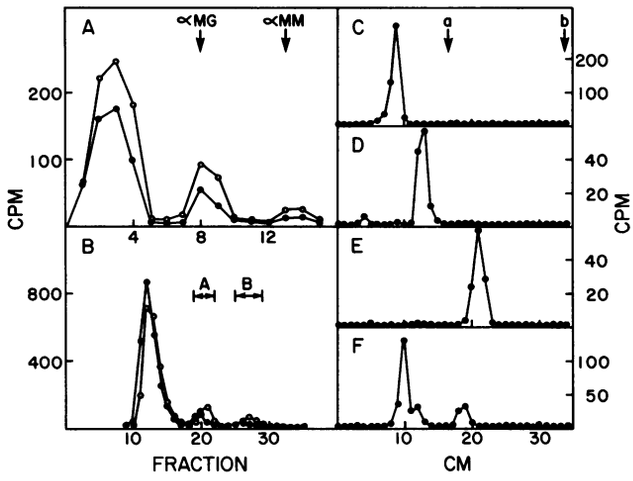

FIG. 2. Analysis of oligosaccharides attached to lgp120 and FcR. (*A*) FcR and lgp120 were isolated from [^{3}H]glucosamine-labeled J774 cells and digested with Pronase (21). The resulting FcR (○) and lgp120 (●) glycopeptides were fractionated on Con A-Sepharose; bound glycopeptides were eluted with 10 mM α-methylglucoside (αMG) and 100 mM α-methylmannoside (αMM). Both lgp120 and FcR glycopeptides had similar chromatographic profiles. (*B*) Glycopeptides isolated from FcR and lgp120 that did not bind to Con A-Sepharose were digested with endo-β-galactosidase [0.5×10^{-3} unit in 0.02 ml of 50 mM sodium acetate (pH 5.9) for 16 hr at 37°C] and the digests were fractionated on a Bio-Gel P-6 column (in 0.1 M ammonium bicarbonate) and pooled as indicated. Endo-β-galactosidase released 17% and 19% of the radioactivity from lgp120 and FcR, respectively. (*C*) The released material fractionated as two peaks, the larger of which (pool A) migrated as a single species during paper chromatography in ethyl acetate/pyridine/acetic acid/water, 5:5:1:3 (vol/vol). (*D*) Although pool A bound to QAE-Sephadex, after neuraminidase digestion 81% failed to bind QAE-Sephadex and migrated on paper as a trisaccharide. (*E*) Further digestion with β-galactosidase resulted in its migration as *N*-acetyllactosamine. Thus, the pool A was composed of a single tetrasaccharide: sialic acid-Gal-GlcNAc-Gal. (*F*) The smaller endo-β-galactosidase-released peak (pool B) appeared heterogeneous after paper chromatography; the fastest component migrating as the disaccharide *N*-acetyllactosamine and the slowest forms as tri- and tetrasaccharides. The pool B tetrasaccharide did not bind QAE-Sephadex, suggesting that some molecules terminate with α-galactose rather than sialic acid (20). The endo-β-galactosidase-released material contained 14% of the [^{3}H]glucosamine or 1.2 residues of the hexosamine. The migration of authentic lactose (arrow a) and GlcNAc (arrow b) are indicated.

features of the domain structure is its degree of similarity to chicken LEP100 (11). The two proteins exhibit an overall amino acid identity of 48%, and both seem to be organized into five similar domains. In addition, the eight cysteines in both proteins are in the same relative locations, as well as eight of the glycosylation sites. The presumptive transmembrane domains are each 24 amino acids and are followed by an identical 11-amino acid cytosolic tail. The only major difference occurs in the proline-rich region, which is longer in LEP100 than in lgp120 (32 vs. 26 residues).

DISCUSSION

Several lgps have been identified and partially characterized from a variety of cells and species. While it is not always apparent which of them represent species-specific versions of the same protein, it is clear that individual lysosomes in a single cell can contain two or more distinct lgps. For example, in mouse cell lines both lgp120 and a second major membrane protein, designated lgp110, can be colocalized (refs. 2 and 8 and B.L.G., A.H., and I.M., unpublished data). lgp120 and lgp110 represent different gene products as indicated by a lack of immunological cross-reactivity (8), amino-terminal sequence analysis, and cDNA cloning (unpublished data and see below). Chen *et al.* (3) have also described two proteins of this type in mouse cells, referred to as LAMP-1 and LAMP-2 (3). By testing the cross-reactivities of monoclonal and polyclonal antibodies, it seems likely that lgp120 and LAMP-1 are identical (unpublished data). The sequence similarity between rat lgp120 and avian LEP100 suggests that LEP100 may be the chicken counterpart of lgp120. However, this possibility remains tentative since these two proteins exhibit somewhat different intracellular distributions. In addition to lysosomes, 7–10% of LEP100 is found on the cell surface and in endosomes (4, 27). lgp120, on the other hand, is found only in lysosomes (1, 8) and in a prelysosomal intermediate compartment (28).

Whatever the precise evolutionary relationship between lgp120 and LEP100, a comparison of these two sequences with a partial sequence for lgp110 (B.L.G., A.H., and I.M., unpublished data) yields several interesting conclusions concerning the domain structure, sequence conservation, and structural relationship among lysosomal proteins from related and diverse species. The general features of each protein are shown schematically in Fig. 3.

The organization of lgp110 is clearly reminiscent of the structural features of lgp120 and LEP100. This is especially interesting because, as mentioned above, lgp120 and lgp110 represent distinct gene products in the same species and thus provide evidence for a family of structurally related membrane glycoproteins. However, the cytosolic tail of lgp110 consists of only 10 amino acids and is only 50% identical to that found in both lgp120 and LEP100.

A striking feature of the ectodomain of all three proteins is the high density of glycosylation sites. Our biochemical data (ref. 6 and unpublished data) show that all of the sites are glycosylated. The carbohydrate moieties contain terminally processed oligosaccharides, and the structure of the N-linked sugars is not peculiar to lgps. Neither lgp120 nor lgp110 was found to contain mannose 6-phosphate residues; although lgp120 does not possess any O-linked carbohydrate, O-linked sugars are present on lgp110 (S.A.G., C.A.G., A.H., and

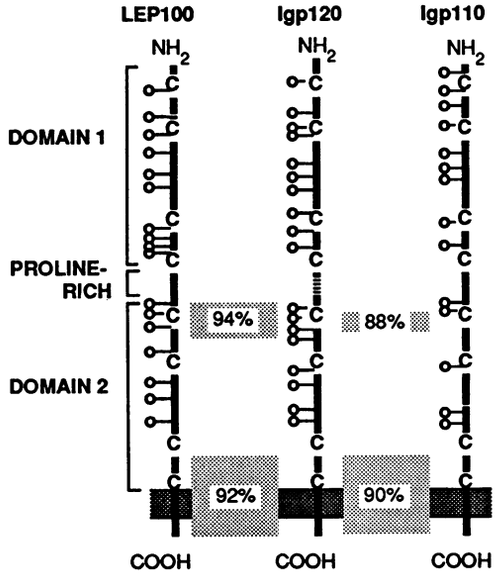

FIG. 3. Comparison of the domain structures of lgp120, lgp110, and LEP100. This diagram emphasizes the similarity of domain organization among these proteins. The luminal domains are divided into three segments. The positions of the eight cysteine residues (C) in domains I and II align upon introduction of gaps (indicated by broken lines). Circles represent sites of asparagine-linked oligosaccharide addition. Gray boxes show percent amino acid similarity [pair scores > 0.5 (29)] in the regions indicated.

I.M., unpublished data). In this regard, it is interesting to note that lgp120 and lgp110 retain terminal sialic residues even after delivery to lysosomes, whereas lysosomal enzymes generally lack sialic acid (30), presumably due to lysosomal acid sialidase activity.

Given the similarity in amino acid sequence of the cytosolic domains of lgp120, LEP100, and lgp110, it is conceivable that all lgp species share common or related cytosolic tails. Whether or not this implies a role in directing the assembly or intracellular transport of these proteins to lysosomes is not yet clear. Similarly, the highly conserved region near the proline-rich domains, or the proline-rich domains themselves, may be involved in specifying functionally important interactions with other luminal or membrane proteins or with other lgps. Although the functions of lgps have yet to be determined, it is already clear that they represent a family of membrane proteins whose degree of structural similarity almost certainly reflects their importance in the biogenesis and/or function of lysosomes in general.

We thank Kathy Stone and Ken Williams for amino acid sequence; Ann Harvey, Victoria Lewis, Heini Miettinen, Helen Plutner, and Mindy Shapiro for their efforts in the work reported here; Janet Emmanuel, Susan Froshauer, and Elisabetta Ullullu for advice and other assistance; and Pat D'Souza and Tom August for anti-LAMP-1 antibodies. This work was supported by research grants from the National Institutes of Health (to I.M., A.H., and C.A.G.), the American Cancer Society (to C.L.H.), and an award from the Swebilius Fund (to B.L.G.).

1. Lewis, V., Green, S. A., Marsh, M., Vihko, P., Helenius, A. & Mellman, I. (1985) *J. Cell Biol.* **100,** 1839–1847.
2. Granger, B. L., Plutner, H., Green, S. A., Mellman, I. & Helenius, A. (1985) *J. Cell Biol.* **101,** 54 (abstr.).
3. Chen, J. W., Murphy, T. L., Willingham, M. C., Pastan, I. & August, J. T. (1985) *J. Cell Biol.* **101,** 85–95.
4. Lippincott-Schwartz, J. & Fambrough, D. M. (1986) *J. Cell Biol.* **102,** 1593–1605.
5. Barriocanal, J. G., Bonifacino, J. S., Yuan, L. & Sandoval, I. V. (1986) *J. Biol. Chem.* **261,** 16755–16763.
6. Ho, M.-K. & Springer, T. A. (1983) *J. Biol. Chem.* **258,** 636–642.
7. Reggio, H., Bainton, D., Harms, E., Coudrier, E. & Louvard, D. (1984) *J. Cell Biol.* **99,** 1511–1526.
8. Green, S., Zimmer, K.-P., Griffiths, G. & Mellman, I. (1987) *J. Cell Biol.* **105,** 1227–1240.
9. Olden, K., Bernard, B. A., Humphries, M. J., Yeo, T.-K., Yeo, K.-T., White, S. L., Newton, S. A., Bauer, H. C. & Parent, J. B. (1985) *Trends Biochem. Sci.* **10,** 78–82.
10. Schauer, R. (1985) *Trends Biochem. Sci.* **10,** 357–360.
11. Fambrough, D. M., Takeyasu, K., Lippincott-Schwartz, J., Siegel, N. R. & Somerville, D. (1988) *J. Cell Biol.* **106,** 61–67.
12. Lathe, R. (1985) *J. Mol. Biol.* **183,** 1–12.
13. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
14. Yanisch-Perron, C., Vieira, J. & Messing, J. (1985) *Gene* **33,** 109–119.
15. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
16. Tabor, S. & Richardson, C. C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4767–4771.
17. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12,** 387–395.
18. Green, S. A., Plutner, H. & Mellman, I. (1985) *J. Biol. Chem.* **260,** 9867–9874.
19. Cummings, R. D. & Kornfeld, S. (1982) *J. Biol. Chem.* **257,** 11235–11240.
20. Cummings, R. D. & Kornfeld, S. (1984) *J. Biol. Chem.* **259,** 6253–6260.
21. Gabel, C. A. & Bergmann, J. E. (1985) *J. Cell Biol.* **101,** 460–469.
22. Young, R. A. & Davis, R. W. (1983) *Science* **222,** 778–782.
23. von Heijne, G. (1986) *Nucleic Acids Res.* **14,** 4683–4690.
24. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132.
25. Baezinger, J. & Kornfeld, S. (1974) *J. Biol. Chem.* **249,** 7270–7281.
26. Kornfeld, S. & Kornfeld, S. (1985) *Annu. Rev. Biochem.* **54,** 631–664.
27. Lippincott-Schwartz, J. & Fambrough, D. M. (1987) *Cell* **49,** 669–677.
28. Griffiths, G., Hoflack, B., Simons, K., Mellman, I. & Kornfeld, S. (1988) *Cell* **52,** 329–341.
29. Gribskov, M. & Burgess, R. (1986) *Nucleic Acids Res.* **14,** 6745–6763.
30. Fedde, K. N. & Sly, W. S. (1985) *Biochem. Biophys. Res. Commun.* **133,** 614–620.