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Identification and Characterization of a Major Lysosomal Membrane Glycoprotein, LGP85/LIMP II in Mouse Liver

Norihiko Tabuchi, Kenji Akasaki,[1] Tomoko Sasaki, Naoko Kanda, and Hiroshi Tsuji

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Fukuyama, Hiroshima 729-02

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We previously have purified and characterized a major lysosomal membrane glycoprotein termed LGP85 (LIMP II) in rat liver lysosomes. In this study, LGP85 in mouse liver lysosomes was identified and characterized by biochemical and molecular biological methods. Lysosomal membranes were isolated from murine liver by differential centrifugation. LGP85 was present in the lysosomal membrane fraction from mouse liver in a comparable amount to another lysosomal membrane glycoprotein, lamp-2. Mouse LGP85 (M-LGP85) from liver lysosomal membranes exhibited an M_r of 80,000 on SDS-PAGE, which is smaller by 5,000 than that of rat LGP85 (R-LGP85). M-LGP85 was immunochemically detected in the extracts of brain, heart, lung, liver, and kidney. A cDNA encoding M-LGP85 was cloned from mouse liver cDNA library. The primary protein structure deduced from a nucleotide sequence of M-LGP85 cDNA indicated that M-LGP85 consists of 478 amino acids with M_r of 54,069. M-LGP85 showed 93.3 and 86.0% sequence similarities to its rat and human counterparts in amino acids, respectively. M-LGP85 contains 11 potential *N*-glycosylation sites which are heavily glycosylated, resulting in the increased M_r of M-LGP85 present in the mouse liver lysosomes. It is likely that M-LGP85 traverses the lysosomal membrane twice, with an NH_2-terminal transmembrane domain, and another hydrophobic domain near the COOH-terminus. M-LGP85 has a protruding COOH-terminal cytoplasmic tail consisting of amino acid residues including the leucine-isoleucine sequence shown to be the lysosomal targeting signal of R-LGP85 and human LGP85 (H-LGP85). The high level of expression of M-LGP85 in the lysosomal membrane, the high structural similarities among M-, R-, and H-LGP85, and the occurrence of M-LGP85 in all the mouse tissues examined suggest the essential and constitutive function of LGP85 in lysosomes.

Key words: cDNA cloning, glycoprotein, lysosome, membrane, mouse.

The limiting membrane of lysosomes has unique highly glycosylated proteins as major constituents. Numerous studies have identified and characterized five families of these glycoproteins, termed lamp-1 (identical to lgp120, LGP107, and LEP100), lamp-2 (identical to lgp110 and LGP96), LGP85 (identical to LIMP II), lysosomal acid phosphatase (LAP) and LIMP I (identical to CD63 and ME491 antigens) [reviewed by Fukuda (*1*), Hunziker and Geuze (*2*), and Perters and von Figura (*3*)]. R-LGP85 was purified from the membrane fraction of rat liver lysosomes by using an immunoaffinity column chromatography with a monoclonal antibody (*4*) and a combination of conventional column chromatographies (*5*). Our previous study (*4*) showed that R-LGP85 is synthesized as a precursor form with an M_r of 77,000 containing 10-12 high-mannose-type *N*-linked oligosaccharide chains. Half of these chains are processed to complex-type chains, resulting in a mature form with M_r of 85,000 in cultured rat hepatocytes. It has been shown that R-LGP85 cycles between the plasma membrane and lysosomes along the endocytic pathway in rat hepatic cells (*6*). cDNAs encoding LGP85 have been cloned from rat and human cells (*7-10*). R-LGP85 shows the high similarities to human LGP85 (H-LGP85) in the deduced amino acid sequences. R- and H-LGP85 retain 11 and 10 putative *N*-glycosylation sites, respectively. Both proteins have two hydrophobic regions, at the NH_2-terminus and near the COOH-terminus, and a protruding COOH-terminal cytoplasmic tail consisting of 20 amino acid residues. The leucine-isoleucine sequence in the tail has been shown to be the lysosomal targeting signal of H-LGP85 (*11*) and R-LGP85 (*12*). However, the function of LGP85 is still unknown.

Thus, a body of information on LGP85 has been accumulated but little is known about LGP85 in mouse cells. It is unclear whether mouse LGP85 (M-LGP85) is indeed present in the lysosomal membrane together with lamp-1 and lamp-2. If it is, comparative studies of the properties and expression levels of M-, R-, and H-LGP85 could provide further information on the mechanism of lysosomal targeting of LGP85 and a clue to understanding its cellular function. In the present study, we first identified and characterized M-LGP85 in the lysosomal membrane fraction prepared from mouse liver. We then cloned a cDNA for

[1] To whom correspondence should be addressed.

Abbreviations: lamp-1 and 2, lysosome-associated membrane glycoproteins 1 and 2, respectively; LIMP II, lysosomal integral membrane protein II; LGP85, lysosomal membrane glycoprotein with M_r of 85,000.

M-LGP85 from a mouse liver cDNA library, deduced the primary structure of M-LGP85 from the nucleotide sequence of M-LGP85 cDNA, and compared it with those of R- and H-LGP85.

MATERIALS AND METHODS

Materials—Male DDY mice (5-10 weeks old) and male Wistar rats (5 weeks old) were obtained from Shimizu Experimental Animal (Kyoto). [α-^{32}P]dCTP was purchased from ICN (Costea Mesa, USA). Restriction enzymes and other DNA modifying enzymes were from Takara Shuzo (Kyoto), Nippon Gene (Toyama), or New England Biolabs. Oligonucleotides for primers of PCR were synthesized and provided by Takara Shuzo. Anti-R-LGP85 polyclonal antibody was raised in white rabbits by injections of R-LGP85 purified from rat liver lysosomal membrane as described previously (*4*). Anti-rat-lamp-2 polyclonal rabbit antibody prepared in the previous study (*13*) was used.

Preparation of Lysosomal Membrane from Mouse Liver—All operations were carried out at 4°C according to the modified method of Ohsumi *et al.* (*14*), which had been developed for the preparation of lysosomal membranes from rat liver. Mice were killed by decapitation, and livers were immediately removed and washed in ice-cold 0.25 M sucrose (pH 7.0) containing 0.2 M KCl (sucrose solution). The livers (30 g wet weight) were minced with scissors and homogenized in an equal volume of sucrose solution using a Physcotron motor-driven homogenizer (NITION, Tokyo; model NS610). The sucrose solution was added to the homogenate, resulting in a 25% homogenate mixture. This homogenate was centrifuged at 650×*g* for 10 min. All centrifugal forces were given on the basis of the average radial distances of tube rotors of the centrifuge. The resultant pellets were resuspended in 30 ml of the sucrose solution using a Potter-Elvehjem homogenizer with three strokes by hand. The suspension was centrifuged at 650×*g* for 15 min. The supernatants obtained from the above two centrifugations were combined, then centrifuged at 11,000×*g* for 20 min. The pellets were suspended in 100 ml of the sucrose solution using a loosely fitted Teflon homogenizer by hand, then centrifuged at 11,000×*g* for 20 min. The supernatant and the fluffy layer over the pellets were discarded. The resultant pellets (mitochondrial and lysosomal fraction; ML fraction) were suspended in 60 ml of the osmotic solution (0.025 M sucrose containing 0.02 M KCl), allowed to stand for 30 min at 0°C, then centrifuged at 11,000×*g* for 20 min. The resultant supernatants were collected carefully without disturbing the fluffy layer covering the well-packed pellets. The pellets were suspended again in 30 ml of the osmotic solution and centrifuged at 11,000×*g* for 20 min. The supernatants were collected carefully as described above. The resultant supernatants were combined. A 1 M $CaCl_2$ solution was added to the supernatants to give a final concentration of 10 mM, and the mixture was allowed to stand for 30 min at 0°C, resulting in the aggregation of contaminating microsomes. The aggregates were pelleted by centrifugation at 5,000×*g* for 15 min, then the resultant supernatants (total lysosomal fraction) were centrifuged at 50,000×*g* for 30 min. The pellets obtained after the final centrifugation were used as lysosomal membranes in the following experiments.

Enzyme Assay—Acid phosphatase was assayed with *p*-nitrophenyl phosphate as a substrate (*15*). Alkaline phosphatase was measured with *p*-nitrophenyl phosphate as a substrate according to the method of Ikehara *et al.* (*16*). One unit of acid phosphatase and alkaline phosphatase activities was defined as the amount releasing 1 μmol of *p*-nitrophenol per min. Glucose-6-phosphatase activity was determined by the method of Swanson (*17*). One unit of glucose-6-phosphatase was expressed as the amount releasing 1 μmol of P_i per min. Catalase activity was measured by the procedure of Beers and Sizer (*18*). Cytochrome oxidase activity was measured spectrophotometrically by the oxidation of reduced cytochrome *c* at 550 nm at 25°C as described by Cooperstein and Lazarow (*19*).

Protein Determination—Proteins were determined by the method of Lowry *et al.* (*20*) using bovine serum albumin as the standard.

Preparation of Tissue Extracts—Tissues were obtained from bled mice and washed extensively in 20 mM Tris-HCl buffer containing 0.15 M NaCl. Twenty percent tissue homogenates were prepared in 20 mM Tris-HCl buffer (pH 7.0) containing 0.5% sodium deoxycholate, 1% Lubrol PX, 1 mM PMSF, 1 μg/ml pepstatin, and 1 μg/ml of leupeptin using a Physcotron motor-driven homogenizer. The homogenates were centrifuged at 105,000×*g* for 1 h. The resultant supernatants were used for immunoblotting.

Gel Electrophoresis—Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 12% slab gels according to Laemmli (*21*).

Immunoblot Procedures—Proteins in SDS-slab gel were transferred to a nylon membrane by electrophoresis, using a modified procedure of Towbin *et al.* (*22*). The positive bands were visualized by means of ECL Western blotting detection kits (Amersham, Bucks, UK) containing a sensitive chemiluminescent substrate for HRP.

Construction of cDNA Library from mRNA of Mouse Liver—Total RNA was prepared from mouse liver by the acid guanidium thiocyanate/phenol-chloroform method (*23*). Poly(A)$^+$ RNA was isolated from the total RNA by chromatography on an oligo(dT)-cellulose column. Double-stranded cDNA was synthesized from poly(A)$^+$ RNA by the method of Gobular and Hoffman (*24*) and ligated with *Eco*RI adaptor after methylation by *Eco*RI methylase. The cDNA library was constructed by inserting *Eco*RI-adaptor-ligated double strand cDNA into λgt11 at the *Eco*RI site.

Preparation of Probe—A part of cDNA encoding R-LGP85 (nucleotides 652-1247 in Ref. 7) was synthesized by the reverse transcription-mediated polymerase chain reaction (RT-PCR) and used as the probe for Northern blotting and plaque hybridization. Poly(A)$^+$ RNA was prepared from the total RNA of rat livers as described above. The first strand cDNA as the template of PCR was synthesized from oligo(dT)-primed poly(A)$^+$ RNA of rat liver by avian myeloblastosis virus reverse transcriptase. The resultant single-stranded cDNA and each primer were denatured at 94°C for 1 min, annealed at 55°C, and used for synthesis of the probe DNA. The synthetic reaction was performed at 68°C for 1 min by *Taq* DNA polymerase in 20 mM Tris-HCl (pH 8.8) containing 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 mg/ml gelatin, and 50 μM deoxynucleotide triphosphates. These reactions were repeated 50 times. An approximately 620-bp fragment obtained by the RT-PCR was labeled with ^{32}P.

cDNA Cloning and Sequencing—cDNA clone for M-LGP85 was isolated from 2×10^5 recombinant phages by the method of Benton and Davis (*25*). Plaque hybridization was carried out with the ^{32}P-labeled cDNA at 65°C in 50 mM Tris-Cl containing 1 M NaCl, 10 mM EDTA, 0.1% sodium *N*-laurosylsarcosinate, 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.2% bovine serum albumin, and 100 μg/ml salmon sperm DNA. The filters were washed twice at 65°C for 30 min in 0.1×SSC (1×SSC=0.15 M NaCl and 0.015 M sodium citrate) and 0.1% sodium *N*-laurosylsarcosinate. Positive clones were purified by four rounds of plaque purification and inserted cDNA were subcloned into the pUC118 plasmid vector at the *Eco*RI site. The nucleotide sequence was determined by the dideoxy-termination method using an ALF automatic sequencer (Pharmacia Biotec, Sweden).

Northern Blot Analysis—Poly(A)$^+$ RNA was denatured, electrophoresed on 0.75% agarose gel containing 2.2 M formaldehyde, then transferred to nitrocellulose filter paper as described by Maniatis *et al.* (*26*). The RNA-transferred filter paper was hybridized with the ^{32}P-labeled R-LGP85cDNA fragment prepared above. Mouse rRNAs were used as the size markers.

RESULTS

Preparation of Mouse Liver Lysosomal Membranes—Lysosomal membranes were prepared from homogenate of mouse livers by differential centrifugation according to the modified method of Ohsumi *et al.* (*14*). The specific activity of LAP in the mouse liver lysosomal membrane was approximately 10 times higher than that in the mouse liver homogenate (Table I). The specific activities of cytochrome oxidase and catalase were barely elevated after the differential centrifugation. Alkaline phosphatase was significantly enriched in the lysosomal membrane fraction of the mouse liver. Such an increase of the specific activity of the plasma membrane marker enzyme is also observed for the lysosomal membranes of rat liver (*14, 29*). The relative specific activity of LAP in the mouse liver lysosomal membrane was about 10 times lower than that in the rat liver one (*14*). LAP occurs in the membrane and content fractions of lysosomes in different protein forms (*3, 27, 28*). Approximately 70% of the total LAP in the rat liver lysosome is recovered in the membrane fraction (*29*). The total lysosomal fraction contained 2.1 units of LAP activity per g of wet liver weight. After separation of lysosomal membranes and contents by centrifugation (see "MATERIALS AND METHODS"), 0.23 unit of LAP activity/g of wet liver weight were recovered in the lysosomal membrane fraction, indicating that only 10% of the lysosomal LAP was localized in the membrane fraction in mouse liver. The lower specific activity of LAP is probably ascribable to the lower distribution ratio of the membrane to the content for LAP in the sub-lysosomal fractions. Therefore, it is unlikely that LAP is appropriate as the maker enzyme for the lysosomal membrane of mouse liver. Our previous work (*13*) has shown that the specific content of lamp-2 in the rat liver lysosomal membrane is 250 times higher than that in the homogenate. Additionally, an immunocytochemical study has shown that mouse lamp-2 is localized exclusively in the lysosomal compartment (*30*). So, we employed lamp-2 as another marker for lysosomal membrane to evaluate the purity of the mouse liver lysosomal membrane obtained here. Lamp-2 in the mouse liver homogenate and lysosomal membranes was readily detected as a protein

TABLE I. **Enzymic composition of mouse liver lysosomal membranes.** Relative enrichment was defined as the ratio of specific activity of the lysosomal membrane to that of the homogenate. Specific activity is expressed as unit/mg protein. Values given are means±SD of three experiments. N.D., not detectable.

Enzyme	Specific activity	Yield (% of homogenate)	Relative enrichment
Protein		0.051±0.01	
Acid phosphatase	1.2±4.0	0.412±0.15	9.93±0.55
Glucose-6-phosphatase	0.079±0.052	0.280±0.19	1.12±0.79
Alkaline phosphatase	0.16±2.9	0.16 ±0.05	3.16±0.32
Cytochrome oxidase	0.066±0.024	0.052±0.02	0.96±0.34
Catalase	N.D.	—	—

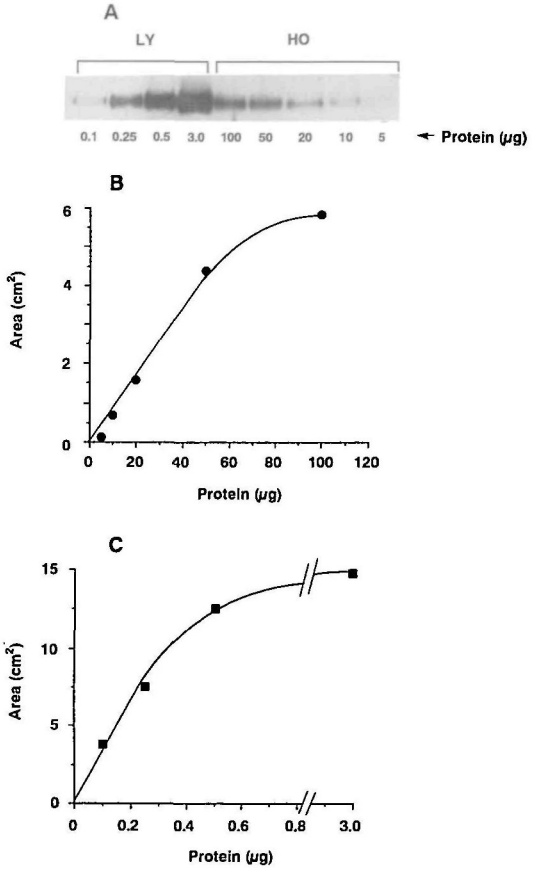

Fig. 1. **Immunoblot analysis of mouse lamp-2 in mouse liver homogenate and lysosomal membrane fractions.** Various amounts of the homogenate (HO) and lysosomal membrane (LY) proteins were electrophoresed on the same slab gel. The separated proteins on the gel were transferred to a nylon sheet by electrophoresis. Thereafter, the nylon sheet was subjected to immunochemical reaction with anti-rat lamp-2 antibody. The immunopositive bands were detected by exposing the nylon sheet to an X-ray film after the chemiluminescent reaction (A). Densities of the bands were quantified by densitometric tracing, and expressed as areas. The areas were plotted against the amounts of the homogenate (B) and lysosomal membrane (C) proteins. The relative enrichment of lamp-2 in the lysosomal membrane is given by the ratio of protein amount of the homogenate to that of the lysosomal membrane at the same area in the linear range.

band with M_r of 110,000 by the chemiluminescent immunoblot procedure using anti-rat lamp-2 antibody (Figs. 1 and 3). Lamp-2 in the mouse liver homogenates and lysosomal membranes was simultaneously quantified by the immunoblotting (Fig. 1). The relative enrichment of lamp-2 from the mouse liver homogenate was calculated to be approximately 350-fold, indicating that lysosomal membrane prepared here from the mouse liver has purity as high as that from the rat liver.

Occurrence of LGP85 in Lysosomal Membranes from Mouse Liver—The mouse and rat liver lysosomal membranes were simultaneously electrophoresed to compare their glycoprotein profiles (Fig. 2). In the rat liver lysosomal membranes, four glycoprotein bands with M_r of 107,000, 96,000, 85,000, and 68,000, which had been identified as lamp-1, lamp-2, LGP85, and LAP, respectively, were stained conspicuously (*4, 13*). In the mouse liver lysosomal membrane, a broad glycoprotein band and a relatively sharp band were intensely stained at M_r positions of 110,000 and 80,000, respectively. The immunoblot analysis data (Fig. 3) indicated that the broad glycoprotein band probably includes lamp-2. When the glycoprotein profiles of the rat and mouse liver lysosomal membranes were compared, the mouse version of LGP85 appeared to be a glycoprotein with M_r of 80,000 (Fig. 2). To confirm this, the rat and mouse liver lysosomal membranes were subjected to immunoblot analysis using anti-rat LGP85 polyclonal antibody. A single positive band migrated to a position of $M_r = 80,000$ in the mouse liver lysosomal membrane, while the rat liver lysosomal membrane provided a positive band of $M_r = 85,000$ (Fig. 3). Additionally, quantitative immunoblot analysis indicated that the specific content of M-LGP85 in the lysosomal membrane is approximately 250 times higher than that in the liver homogenate (data not shown). These results indicated that M-LGP85 is indeed present in the mouse liver lysosomal membrane in a comparable amount to lamp-2.

Immunoblot Detection of M-LGP85 in Mouse Tissues—

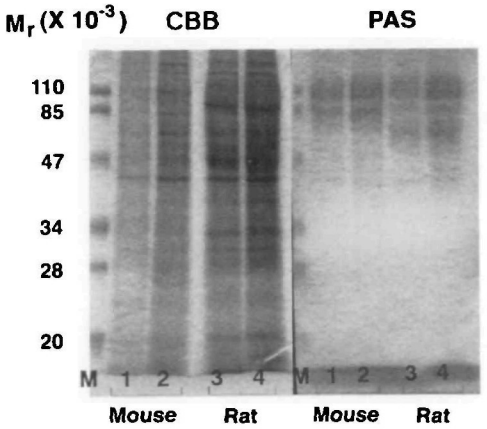

Fig. 2. **SDS-PAGE of lysosomal membrane proteins obtained from mouse and rat livers.** The proteins of lysosomal membrane proteins from the mouse and rat liver homogenates were subjected to SDS-PAGE. Lanes 1 and 2, 10 and 20 μg of mouse liver lysosomal membrane proteins, respectively; lanes 3 and 4, 15 and 30 μg of rat liver lysosomal membrane proteins, respectively. Gels were obtained after Coomassie Blue staining for proteins (CBB) and periodate-Schiff for carbohydrates (PAS). The prestained marker proteins (Bio-Rad, USA) were simultaneously electrophoresed in lane M: phosphorylase *b* (110,000), bovine serum albumin (85,000), ovalbumin (52,000), carbonic anhydrase (34,000), soybean trypsin inhibitor (28,000), and lysozyme (20,000).

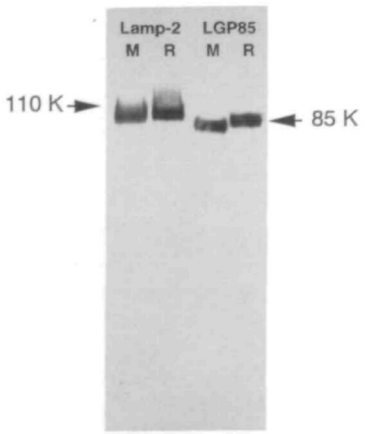

Fig. 3. **Immunoblot analysis of mouse and rat liver lysosomal membranes with anti-rat lamp-2 and anti-rat LGP85 antibodies.** The same amounts of lysosomal membrane proteins from mouse (lane M) and rat (lane R) livers were simultaneously analyzed by immunoblotting with anti-rat lamp-2 and rat LGP85 antibodies. Arrows indicate migration positions of the prestained phosphorylase *b* (110 K) and bovine serum albumin (85 K).

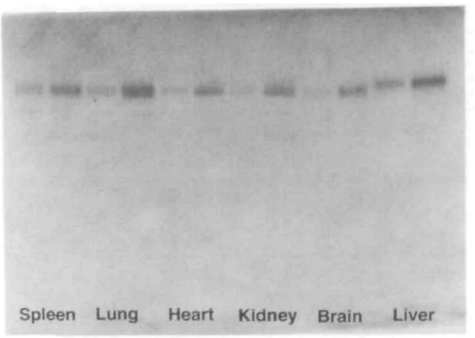

Fig. 4. **Immunoblot analysis of mouse tissues with anti-rat LGP85 antibody.** Appropriate amounts of extracts of the mouse tissues were analyzed by immunoblotting using the anti-rat LGP85 antibody. The protein amount in the left of each pair of lanes is 10 times less than that in the right.

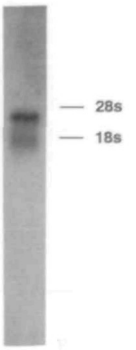

Fig. 5. **Northern blot analysis of RNA from mouse liver.** Poly(A)$^+$ RNA was purified from the total mouse liver RNA and subjected to hybridization with the R-LGP85 cDNA fragment labeled with ^{32}P.

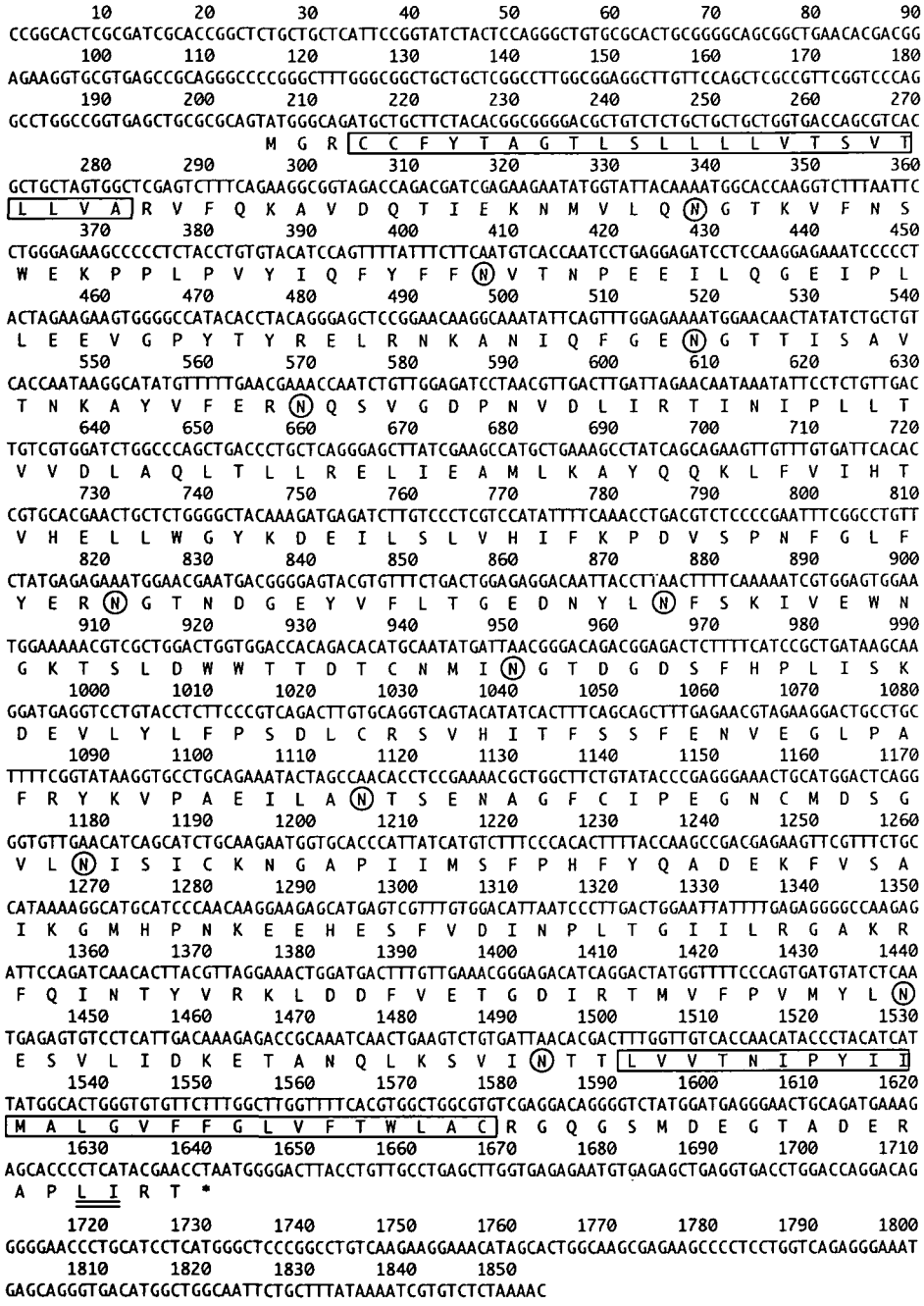

Fig. 6. **Nucleotide sequence of cloned cDNA and deduced amino acid sequence of M-LGP85.** Nucleotides are numbered above the lines. The deduced amino acid sequence is shown below the nucleotide sequence. Sequences in boxes indicate stretches of hydrophobic amino acids, possible *transmembrane domains*, at the NH_2-terminus and near the COOH-terminus. Asparagine residues closed with circles represent potential *N*-linked glycosylation sites. The stop codon limiting the open reading frame is indicated by an asterisk. Doubled underline indicates the leucine-isoleucine involved in lysosomal targeting.

We investigated the presence of M-LGP85 in five mouse tissues including liver by Western blotting (Fig. 4). Immunoreactive bands were detected in all the tissues examined, but the molecular weights of M-LGP85 in extrahepatic tissues were lower than the liver M-LGP85. The different molecular weights might be due to different *N*-glycosylations, as observed for lamp-1 and lamp-2 molecules from various cell types (*13*).

Northern Blot Analysis of Transcripts of M-LGP85 Gene—When poly(A)$^+$ RNA from mouse liver was analyzed by Northern hybridization using a part of R-LGP85 cDNA as a probe, two RNA bands were found corresponding to sizes of 3.7 and 1.8 kb (Fig. 5). The 1.8-kb transcript is in agreement with the size of cDNA isolated below. The two forms of mRNA probably arise by the use of different 3′-untranslated regions by alternative splicing. The explanation of this observation will require structural analysis of the 3.7-kb transcript.

Nucleotide Sequence of cDNA Encoding M-LGP85—A λgt11 cDNA library constructed from mouse liver was screened by plaque hybridization with the ^{35}P-labeled R-LGP85 cDNA fragment, and two positive clones were isolated from about 2 $\times 10^5$ plaques. One of the two was subjected to nucleotide sequencing, and the sequence of the coding region is shown in Fig. 6. The cloned M-LGP85 cDNA contained an entire coding region of M-LGP85 (nucleotides 206-1639) flanked by 5′-untranslated region of 205 nucleotides and a 3′-untranslated region of 214

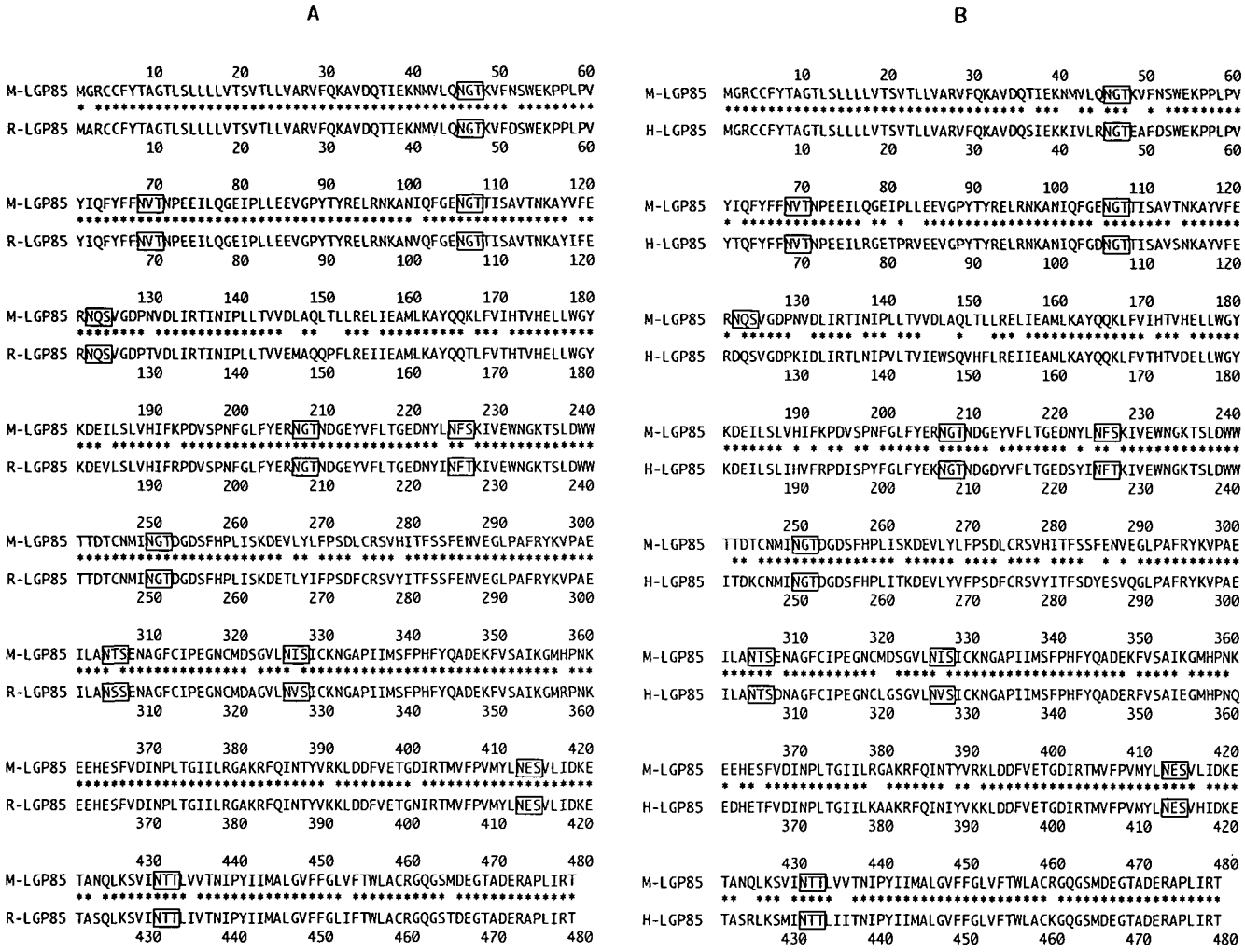

Fig. 7. **Alined amino acid sequences of M-LGP85 and R-LGP85 or H-LGP85.** M-LGP85 shows 93.3 and 86.0% sequence similarities to R-LGP85 (A) and H-LGP85 (B), respectively. Boxes indicate potential *N*-linked glycosylation sites. Asterisks indicate identical residues.

nucleotides. The M-LGP85 cDNA potentially encodes a 478-amino acid polypeptide, starting from the first initiation codon (ATG). The molecular weight of the polypeptide including the signal peptide was calculated to be 54,069. There are 11 potential *N*-glycosylation sites (Asn-X-Thr/Ser) in the primary structure of M-LGP85. The number and the positions of these glycosylation sites in M-LGP85 are the same as those in R-LGP85, although H-LGP85 has 10 sites because Asn at position 122 is converted to Asp (Fig. 7). M-LGP85 possesses two hydrophobic regions at the NH_2-terminus and near the COOH-terminus, and the 20 amino acid cytoplasmic tail including the COOH terminal amino acid.

DISCUSSION

The present study involved the identification and characterization of M-LGP85 by biochemical and genetic procedures. The method developed for isolation of rat liver lysosomal membranes was applied to the preparation of the lysosomal membranes from mouse liver and yielded a highly purified lysosomal membrane fraction as judged by the enrichments of the lysosomal marker enzyme, acid phosphatase, and the lysosome-associated membrane glycoprotein, lamp-2. A highly glycosylated protein band with M_r of 80,000 was detected in the mouse liver lysosomal membrane, and this protein reacted with anti-R-LGP85 polyclonal antibody. The cross-reaction between M- and R-LGP85 with the antibody is concomitant with the high similarity of LGP85 from two different mammals in the primary structure deduced (93.3% sequence similarity as shown in Fig. 7). M_r of hepatic lysosomal M-LGP85 is less by 5,000 than that of hepatic lysosomal R-LGP85. Since the core proteins of M- and R-LGP85 have almost the same molecular weight (Fig. 7), it is likely that mouse hepatic LGP85 has lower contents of carbohydrates than rat hepatic LGP85.

R-LGP85 represents approximately 4% of the total proteins of rat liver lysosomal membranes (*4*). The carbohydrate staining of M-LGP85 is as much intense as that of R-LGP85 when the lysosomal membrane proteins from these two sources are electrophoresed under similar conditions (Fig. 2). Thus, M-LGP85 seems to account for more than 4% of the total lysosomal membrane proteins of mouse liver. The high level of expression of M-LGP85 in the lysosomal membrane, the high structural similarities

among M-, R-, and H-LGP85, and the occurrence of M-LGP85 in all the mouse tissues examined suggest the essential and constitutive function of LGP85 in lysosomes. One of the constitutive lysosomal functions is degradation of intracellular proteins through the autophagic process. Cuervo and Dice (*31*) have recently shown that lamp-2 serves as a receptor for selective uptake and degradation of cytosolic RNase A and glyceraldehyde-3-phosphate dehydrogenase.

The cytoplasmic tail of lamp-2 is critical for the binding of the cytosolic proteins. The cytoplasmic tail of lamp-2 is critical for the binding of the cytosolic proteins. Since LGP85 is a constitutive membrane protein of lysosomes and contains a cytoplasmic tail as well as lamp-2, it might be involved in sequestration of particular cytoplasmic proteins destined for lysosomal degradation.

It has been shown that R-LGP85 traverses the membrane twice, at the NH_2-terminus and near the COOH-terminus (*7*). An NH_2-terminal amino acid of R-LGP85 is Ala next to the initial Met, indicating that R-LGP85 has an uncleavable signal peptide which might be included in the first membrane-anchoring domain. Although additional studies are required to determine whether M-LGP85 indeed retains the NH_2-terminal membrane-anchoring domain, the very high similarities between M- and R-LGP85 in the NH_2-terminal 30 amino acid sequence, as shown Fig. 7, suggest that M-LGP85 is integrated in the lysosomal membrane with the two hydrophobic regions in the same way as R-LGP85.

It is generally accepted that lysosomal membrane glycoproteins are synthesized in rough endoplasmic reticulum and then transferred to the *trans*-Golgi network (TGN) through the Golgi apparatus, where their carbohydrate chains are modified (*32*). After lamp-1 and lamp-2 leave TGN, they are transported to lysosomes along the endocytic compartments in a complex manner (*33-35*). The biosynthetic pathway of LGP85 is understood less well than those of lamp-1 and lamp-2, although biochemical and immunocytochemical studies using the cells expressing R- and H-LGP85 mutants revealed that the Leu-Ile sequence in the cytoplasmic tail is critical for the LGP85 localization to lysosomes (*11, 12*). Additionally, the amino acid sequence of the tail in M-LGP85 is identical to that in R-LGP85 and almost the same as that in H-LGP85 (Fig. 7). Taken together, these results suggest that M-LGP85 is also delivered to the lysosomal membrane by the cellular machinery that recognizes the Leu-Ile motif located appropriately in the cytoplasmic tail.

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