The pH-dependent Membrane Association of Procathepsin L Is Mediated by a 9-Residue Sequence within the Propeptide*

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The lysosomal protease procathepsin L binds to mouse fibroblast microsomal membranes at pH 5, but mature active cathepsin L does not (McIntyre, G. F., and Erickson, A. H. (1991) J. Biol. Chem. 266, 15438-15445). This binding is not dependent on N-linked carbohydrate as procathepsin L synthesized in cells treated with tunicamycin still shows pH-dependent membrane association. These results suggest that the propeptide (Thr16-Lys115) of the cysteine protease mediates its pH-dependent membrane association. Synthetic peptides containing either 24 or 9 residues from the N-terminal portion of the mouse procathepsin L propeptide inhibited the binding of mouse procathepsin L to microsomal membranes at pH 5. In contrast, the pH-dependent membrane association was not inhibited either by a scrambled version of the 24-residue peptide, in which 11 N-terminal residues from mature mouse cathepsin L. The 24-residue peptide chemically coupled to horseradish peroxidase bound to microsomes at pH 5, but not at pH 7. On ligand blots, the same conjugate bound specifically to a 43-kDa integral membrane protein, identifying the microsomal protein that mediates the proenzyme binding. The 9-residue propeptide sequence that inhibits the membrane association of procathepsin L at pH 5 resembles the vacuolar sorting sequences in the propeptides of yeast proteinase A and carboxypeptidase Y. This suggests that the membrane association of procathepsin L may play a role in the transport of the proenzyme to lysosomes, the vacuolar equivalent in mammalian cells.

Two mouse lysosomal proenzymes, procathepsins L and D, bind to microsomal membranes at pH 5, but the active forms of these proenzymes do not (1). This membrane association is independent of the mannose 6-phosphate receptors (MPR)1, which are involved in the cellular targeting of soluble lysosomal enzymes (1). Several other mammalian lysosomal proteins have been found to exhibit MPR-independent binding to membranes. Procathepsin D is membrane-bound in macrophage endosomes (2), glucocerebrosidase, prosaposin, and procathepsin D bind to microsomal membranes in hepatoma cells (3, 4), and procathepsin C is membrane-bound during transport to lysosomes in a cell line lacking the cation-independent MPR (5). Similarly, Dictyostelium discoideum proenzymes, which are sorted to lysosomes by an MPR-independent pathway (6), are membrane-bound, but the active proteases are not (7). The physiological function of the membrane association of these proteins is not clear and the receptor proteins mediating their membrane association have not been identified.

The fact that the proenzymes but not the active mature forms bind to membranes suggests that the propeptides of lysosomal and vacuolar enzymes not only block their proteolytic activity but also mediate their MPR-independent membrane association. Two yeast proenzymes, proteinase A (8) and carboxypeptidase Y (CPY) (9-11), possess short amino acid sequences in their propeptides which are thought to interact with presently unidentified receptor(s) that mediate MPR-independent sorting to vacuoles. Receptor binding may be regulated by pH, as it is in mammalian cells, because yeast strains that cannot maintain acidic vacuoles secrete precursor forms of CPY and proteinase A (12). Similarly, in Dictyostelium the N-terminal 70 amino acids of B-hexosaminidase are required for sorting of this enzyme to vesicles of intermediate density, prior to delivery to lysosomes (13).

In the present study, we show that two synthetic peptides based on sequences from the propeptide of mouse procathepsin L inhibit binding of procathepsin L to microsomal membranes at pH 5. The membrane association of procathepsin L at pH 5 may be mediated by a 6-residue propeptide sequence that is similar to the propeptide vacuolar-sorting sequences of two yeast proenzymes.

**EXPERIMENTAL PROCEDURES**

**Materials**—Reagents used include [35S]methionine (Tran35S-label; 850-950 Ci/mol) from ICN Biomedica's, Costa Mesa, CA; Dubesco's modified Eagle's medium, Opti-MEM, and fetal bovine serum were from Life Technologies, Inc.; phenylmethanesulfonyl fluoride, aprotinin, pepstatin, the cysteine protease inhibitor E-64, &-thiogalactoside, and HEPES were from Boehringer Mannheim; ECL Western Blotting Detection Reagents and donkey anti-rabbit serum conjugated to horseradish peroxidase were from Azizhams Corp.; the HotBox System was from Billups-Rothenberg, Del Mar, CA; and Immobilon-P was from Millipore, Bedford, MA. All other reagents were the highest quality available and were obtained from either Sigma or Fisher.

**Cells**—The Kirsten sarcoma virus-transformed NIH 3T3 cell line (KNIH) was a gift of Dr. C. Scher, University of Pennsylvania. Cells were grown in Dulbecco's modified Eagle's medium containing 4.5 g/liter of glucose, 10% fetal bovine serum, 0.005% gentamycin, 0.0025% kanamycin, and 200 µg/ml of glutamine. Radiolabeling—Procathepsin L was radiolabeled by washing cell monolayers twice with phosphate-buffered saline (pH 7.2), starving for 1 h in Dulbecco's modified Eagle's medium lacking methionine, but containing 8% dialyzed fetal bovine serum, 200 µg/ml glutamine, 75 units/ml of streptocin, and 75 units/ml of penicillin G, and pulsing in this cell starvation medium supplemented with [35S]methionine (500 µCi/ml) in a Billups-Rothenberg HotBox for the times indicated.

**Microsomal Membranes**—Microsomal membranes were prepared from KNIH mouse fibroblasts by a modification of the method of Diment.
et al. (2) as described by McIntyre and Erickson (1). When specified, the membranes were prepared from cells treated with tunicamycin (1.5 μg/ml culture medium) for 8 h.

Membrane Binding—Microsomal membranes were prepared from one 85-mm dish of unlabeled KNH cells as described by McIntyre and Erickson (1). The membranes were stripped of bound procathepsin L and other peripheral proteins by resuspending the pellet in 30 mM sodium bicarbonate buffer (pH 10.6) (14, 15) containing 10 mM mannose 6-phosphate, 1 mM EDTA, and 1 mM EGTA, plus 0.5% saponin to produce stable membrane holes (16). In addition, the membranes were lysed by two cycles of freezing and thawing followed by homogenization to ensure that membrane binding was susceptible to saponin were opened. The stripped membranes were pelleted by centrifugation at 120,000 × g for 15 min at 4 °C, and the membrane pellet was resuspended in 100 μl of water.

The binding reaction mixture consisted of the membrane suspension (20 μl), a synthetic peptide (as specified), pH 5 binding buffer (130 μl) containing 30 mM sodium acetate, 10 mM mannose 6-phosphate, 1 mM EDTA, 1 mM EGTA, 1 mM phenylmethanesulfonyl fluoride, 1 μM pepstatin, 1 μM E-64, 0.5% saponin, 1 mg/ml of bovine serum albumin, and cell culture medium (50 μl) containing [35S]methionine-labeled procathepsin L, which had been obtained from one 85-mm plate of KNH cells that were cultured in starvation medium containing 5% fetal calf serum (13). For binding, samples were incubated at 4 °C for 1.5–2 h in an Eppendorf mixer. Microsomes were collected by centrifugation at 120,000 × g for 15 min at 4 °C. Cathepsin L was immunoprecipitated from the supernatants and from the pelleted microsomal membranes using polyclonal rabbit antisera (17) and quantitated as described (1).

When a synthetic peptide was used in binding studies was peptide A conjugated to horseradish peroxidase, the peroxidase was visualized by chemiluminescence.

Synthetic Peptides—Peptides were synthesized by the solid-phase method and purified by reverse-phase HPLC at the University of North Carolina, Chapel Hill/National Institute of Environmental Health Sciences Protein Chemistry Laboratory. When specified, peptides were alkylated for 2 h at room temperature with 0.12 M iodoacetamide in 0.25 M Tris-HCl buffer (pH 7.0) or 50 mM sodium hydroxide containing 66 mM dithiothreitol, 0.25 M Tris (pH 7.5) and 10 mM EDTA and desalting on Sephadex G10. Following alkylation, the peptides were again desalted and purified by HPLC.

Ligand Blots—KNH integral membrane proteins were resolved on a 15%–30% polyacrylamide gel in the presence of SDS (18) and electrophoretically transferred to Immobilon-P in 10 mM CAPS containing 10% methanol (v/v) using a semi-dry Western blotting apparatus (Integrated Separation Systems, Hyde Park, MA) at 1 mA/cm² for 1.5 h. Free binding sites on the blot were blocked by incubating the blot with 10 μl Tris-HCl buffer (pH 7.4) containing 150 mM NaCl and 0.05% Tween 20 (TWEEN-saline) plus 5% Carnation non-fat dry milk for 2 h at room temperature. The blot was then incubated for 4 h with constant agitation with 500 μl of a blocking solution containing 0.12 μM [35S]methionine-labeled procathepsin L, pH 5 binding buffer, and 1 mg/ml of bovine serum albumin. The blot was then washed five times for 30 min in the same buffer used for binding. Bound peptide was detected by chemiluminescence on exposure of the blot to x-ray film for 30 to 3 min. The amount of conjugate in each sample was determined by scanning the autoradiograph with a computing densitometer.

RESULTS

The pH-dependent Membrane Association of Procathepsin L Is Not Dependent upon High-mannose Carbohydrate—We previously determined that the pH 5-dependent membrane association of procathepsin L was independent of mannose 6-phosphate recognition markers (1). In order to determine whether membrane binding requires some other modification of high-mannose carbohydrate, we examined the membrane association of procathepsin L in KNH cells treated with tunicamycin. Tunicamycin is a nucleoside antibiotic which blocks the formation of N-acetylglucosamine-lipid intermediates which serve as donors for the synthesis of high-mannose carbohydrate structures (20). Procathepsin L synthesized in cells treated with the drug is approximately 2 kDa smaller than authentic procathepsin L (Fig. 1, lanes 1 and 2), consistent with failure to acquire its single carbohydrate chain cotranslationally. This 34-kDa form of procathepsin L remained membrane-bound and was not released to the supernatant when microsomal membranes were prepared and lysed at pH 5 (Fig. 1, lane 3). In contrast, most of the procathepsin L was released to the supernatant when the same membranes were subsequently washed at pH 7 (Fig. 1, lane 4). Additional procathepsin L was solubilized when the membranes were washed at pH 10.6 (Fig. 1, lane 5), which releases peripheral membrane proteins (14, 15). These results are similar to those obtained with glycosylated procathepsin L (1). Essentially all of the protein modified with carbohydrate is released from the membranes by the pH 10.6 wash (1). However, when the lysosomal protease lacks high-mannose carbohydrate, some procathepsin L appears to remain with the membranes even after this wash (Fig. 1, lane 6), perhaps due to the presence of aggregates of the nonglycosylated protein which pellet with the membranes.

Thus the high-mannose carbohydrate on procathepsin L is not mediating the pH-dependent membrane association. Consistent with this finding, the propeptide does not contain N-linked carbohydrate and neither 10 nm mannose 6-phosphate nor 50 mM mannose released procathepsin L from microsomal membranes at pH 5 (1).

Synthetic Peptide A Containing the N-terminal 24-Residue Sequence of Procathepsin L Inhibits Binding of Procathepsin L to Membranes at pH 5—We noted that even after it had been denatured, reduced, and alkylated, cellular procathepsin L still bound to microsomal membranes at pH 5, which suggested that a linear sequence of amino acids, not a conformational determinant, was sufficient for binding. We therefore determined whether or not synthetic peptides from the N terminus of the procathepsin L propeptide could compete with the proenzyme for binding to microsomal membranes at pH 5. We first assayed peptide A consisting of the N-terminal 24 amino acid residues (Thr18-Gly41) of mouse procathepsin L (which lacks the signal peptide but contains the 96-residue N-terminal propeptide) plus alanyl-cysteine amide (Fig. 2). Peptide A inhibited the binding of procathepsin L to microsomal membranes at pH 5 in a reproducible and dose-dependent manner. Binding of radiolabeled procathepsin L to KNH microsomal membranes stripped of peripheral membrane proteins was inhibited by 50% when 10 μM peptide A was present (Fig. 3). The same results were obtained whether or not peptide A was alkylated.

2 G. F. McIntyre and A. H. Erickson, unpublished data.
N-terminal 11 residues (Ile114-Gly124) of mouse cathepsin L. The procathepsin L sequence (17) is numbered from the initiator Met. The C-terminal Ala-Cys-NH$_2$ residues (underlined) were added during peptide synthesis to facilitate iodination and/or resin attachment.

These results demonstrate that the N-terminal 24 amino acids of procathepsin L comprise at least a part of the polypeptide site which mediates the binding of the proenzyme to microsomal membranes at pH 5.

**Synthetic Peptide B Containing a 9-Residue Sequence from the Procathepsin L Prepropeptide Inhibits Binding of Procathepsin L to Membranes at pH 5**—In order to determine if the entire sequence of peptide A was necessary for membrane binding, we tested a sequence from the C terminus of the 24-residue peptide A. Peptide B consisted of nine residues (Lys116-Gly124) from mouse procathepsin L plus alanyl-cysteine amide (Fig. 2). Peptide B also inhibited the binding of procathepsin L to microsomal membranes at pH 5 in a dose-dependent manner. Binding of radiolabeled procathepsin L to stripped KNHI microsomal membranes was inhibited by 10% or less even when 250 μM peptide C was present in the conditioned KNHI-cell medium incubated with the membranes (Fig. 3).

**Control Peptide C Containing the N-terminal 11-Residue Sequence from Active Cathepsin L Does Not Inhibit Binding of Procathepsin L to Membranes at pH 5**—As a control we tested a synthetic peptide based on the N terminus of active cathepsin L for its ability to inhibit the binding of procathepsin L to microsomal membranes at pH 5. Peptide C consisted of the N-terminal 11 residues (Ile114-Gly124) of mouse cathepsin L plus tyrosine amide (Fig. 2). In contrast to the inhibition observed with peptides A and B, peptide C did not significantly inhibit the binding of procathepsin L to microsomal membranes at pH 5. Binding of radiolabeled procathepsin L to stripped KNHI microsomal membranes was inhibited by 10% or less even when 250 μM peptide C was present in the conditioned KNHI-cell medium incubated with the membranes (Fig. 3).

**Fig. 2. Location of synthetic peptides A, B, and C within mouse procathepsin L.** The procathepsin L sequence (17) is numbered from the initiator Met. The C-terminal Ala-Cys-NH$_2$ residues (underlined) were added during peptide synthesis to facilitate iodination and/or resin attachment.

**Fig. 3. Synthetic peptides A and B inhibit binding of 35S-labeled procathepsin L to microsomal membranes at pH 5.** Equal aliquots of KNHI microsomal membranes stripped of procathepsin L and peripheral membrane proteins by washing at pH 10.6 were incubated with [35S]methionine-labeled procathepsin L and increasing concentrations of peptides A (■), B (▲), C (○), or scrambled peptide A (▲) (Figs. 2 and 4). Peptide A was assayed both with (■) and without (▲) alkylation of the C-terminal Cys residue. Control peptides were assayed in the same experiment as peptides A and B. After 2 h, the membranes were pelleted by centrifugation, and procathepsin L was immunoprecipitated from the pellets. The immunoprecipitated proteins were resolved on 12.5% polyacrylamide gels, and the amount of procathepsin L in each sample was determined by scintillation counting as described previously (1). The percentage of procathepsin L bound to membranes in the presence of peptide relative to the percentage bound in the absence of peptide is plotted versus peptide concentration.

These data demonstrate that the N-terminal 24 amino acids of procathepsin L comprise at least a part of the polypeptide site which mediates the binding of the proenzyme to microsomal membranes at pH 5.

**Fig. 4. Three amino acid residues which are charged at pH 5 are dispersed in a scrambled version of Peptide A.** A scrambled version of peptide A was synthesized in which 3 adjacent residues expected to be positively charged at pH 5 (His36-Arg37-Arg38) were dispersed by exchanging His36 for Thr18, Arg27 for Asp26, and Arg38 for Ala37. The exchanged residues are boxed. The procathepsin L sequence (17) is numbered from the initiator Met. The C-terminal Ala-Cys-NH$_2$ residues added during peptide synthesis to facilitate iodination and/or resin attachment was alkylated prior to inhibition assays. The sequence contained within peptide B, which is homologous to yeast vacuolar sorting sequence (see Fig. 7), is indicated in bold letters.

**Fig. 5. Synthetic peptides A and B inhibit binding of procathepsin L to microsomal membranes at pH 5.** Binding of radiolabeled procathepsin L to stripped KNHI microsomal membranes was inhibited by 10% or less even when 250 μM peptide C was present in the conditioned KNHI-cell medium incubated with the membranes (Fig. 3).

**A Scrambled Version of Peptide A Does Not Inhibit Binding of Procathepsin L to Membranes at pH 5—**As a second control, we tested a scrambled version of peptide A for its ability to inhibit the binding of procathepsin L to microsomal membranes at pH 5. Three adjacent residues in peptide A which are likely to be positively charged at pH 5 (His36-Arg37-Arg38) and which are contained in peptide B were dispersed in the scrambled version of peptide A by exchanging His36 for Thr18, Arg27 for Asp26, and Arg38 for Ala37. No other residues were altered. Like peptide B, scrambled peptide B did not significantly inhibit the binding of procathepsin L to microsomal membranes at pH 5. Binding of radiolabeled procathepsin L to KNHI microsomal membranes stripped of peripheral proteins was inhibited by 10% or less even when 247 μM scrambled peptide B was present (Fig. 3).

**Peptide A Mediates the Binding of a Peptide A-Horseradish Peroxidase Conjugate to Microsomal Membranes at pH 5**—Further evidence that the N terminus of the procathepsin L propeptide is responsible for the pH 5-dependent membrane association of the proenzyme came from direct peptide binding experiments. Peptide A chemically conjugated to horseradish peroxidase mediated the pH-dependent membrane association of this unrelated protein (Fig. 5). Five times more peptide A-horseradish peroxidase conjugate bound to microsomal membranes at pH 5 (panel B, lane 1) than at pH 7 (panel B, lane 2). This is consistent with the results of binding experiments carried out with procathepsin L from cell-conditioned media (1). Horseradish peroxidase alone did not bind to microsomal membranes at pH 5 or at pH 7 (panel A), demonstrating that it is peptide A which is specifically interacting with the membranes in a pH-dependent manner.

**Peptide A Mediates the Binding of a Peptide A-Horseradish Peroxidase Conjugate to a 43-kDa Integral Microsomal Membrane Protein at pH 5**—The peptide A-peroxidase conjugate bound to a 43-kDa integral membrane protein at pH 5 on ligand blots (Fig. 6, lane 1), but did not bind to this membrane protein at pH 7 (Fig. 6, lane 2). Scrambled peptide A chemically con-
jugated to peroxidase did not bind to the 43-kDa protein on ligand blots (data not shown). Similarly, procathepsin L bound to a 43-kDa membrane protein at pH 5 but not at pH 7, and the binding was inhibited by peptide A but not by scrambled peptide A (21). These results suggest that the protein which binds procathepsin L at acidic pH is a specific 43-kDa integral membrane protein.

**DISCUSSION**

*Specificity of Peptide Inhibition*—We have determined that two synthetic peptides based on the N terminus of procathepsin L inhibit the pH 5-dependent association of this protein with microsomal membranes. Two control peptides, a scrambled version of the inhibitory 26-residue peptide and a peptide based on the N terminus of active mature cathepsin L, failed to affect the membrane association. These results indicate that the pH-dependent membrane association of the lysosomal cysteine protease is mediated, at least in part, by its activation peptide. All four synthetic peptides assayed have a net positive charge at pH 5. Peptides A, B, and scrambled peptide A are all +5 and peptide C is +2 at pH 5. By the criterion of net charge per residue at pH 5, peptide C (+0.17) resembles peptide A (+0.19). By the criterion of percentage of aromatic residues (Phe, Trp, Tyr), peptide C (17%) also resembles peptide A (19%). Thus the binding activity of peptide A is not due to its total charge or aromacity alone, but to its specific amino acid sequence.

The apparent binding affinity of peptide A (IC\textsubscript{50} = 10 μM) is about 30 times that of peptide B (IC\textsubscript{50} = 300 μM), indicating that the first 15 residues of peptide A (Thr\textsuperscript{16}–Trp\textsuperscript{25}) increase the binding affinity of the peptide for the membrane. At least 1 of the 3 adjacent residues in peptide B likely to be charged at pH 5 (His\textsuperscript{96}–Arg\textsuperscript{97}–Arg\textsuperscript{98}), and perhaps more than one, is important for the pH 5-dependent membrane binding activity of mouse procathepsin L. Exchanging these 3 residues in peptide B for 3 residues in the first 10 amino acids of peptide A produced a scrambled peptide which no longer inhibited the membrane association of procathepsin L.

The peptide B segment of the mouse cathepsin L proprotease may be located between two α-helices. Helical wheel analysis indicates that the sequence N-terminal to the peptide B segment may form an α-helix with a hydrophobic face comprised of Phe\textsuperscript{21}, Phe\textsuperscript{25}, Trp\textsuperscript{29}, and Trp\textsuperscript{32}. A second potential α-helix starts 5 residues after Leu\textsuperscript{39}, the hydrophobe of the peptide B segment. Residues Glu\textsuperscript{44}, Arg\textsuperscript{48}, Ile\textsuperscript{51}, Asn\textsuperscript{52}, Ile\textsuperscript{56}, and Asn\textsuperscript{57} comprise the ERFNIN motif, a highly conserved interspersed motif of unknown function in the propeptide segment of all cysteine proteases except the cathepsin B subgroup (22). Helical wheel analysis of Glu\textsuperscript{44} to Asn\textsuperscript{56} indicates that these 6 conserved residues are also likely to lie on the same face of an α-helix (22).

The pH-dependent membrane association is not mediated by the C-terminal Cys residues added to the peptides during synthesis to facilitate resin attachment. The inhibition curve for peptide A was essentially the same whether or not the peptide was alkylated (Fig. 3). These results support our hypothesis (1) that the binding of procathepsin L to microsomal membranes at pH 5 is not due to random association with various membrane proteins or lipids but to a specific interaction with an integral membrane protein which is mediated by the propeptide of procathepsin L.

The 9-Residue Propeptide Sequence That Inhibits Binding of Procathepsin L to Membranes at pH 5 Resembles Yeast Vacuolar-sorting Sequences—We specifically selected the 9-residue propeptide sequence present in peptide B for testing because it resembles (Fig. 7) the propeptide sequences that direct the sorting of two yeast proproteins to vacuoles, which are functionally equivalent in unicellular eukaryotes to mammalian lysosomes. A short sequence within the propeptide of yeast CPY sorts this propeptide to vacuoles (9, 10). By site-directed mutagenesis, 4 contiguous amino acid residues (Gln\textsuperscript{24}–Arg\textsuperscript{25}–Pro\textsuperscript{26}–Leu\textsuperscript{27}, prepro-CPY numbering) were determined to be essential for the correct sorting of pro-CPY to vacuoles (11). Mutation of any of these residues results in secretion of the mutant proprotease, although these 4 residues may not constitute the entire vacuolar-sorting sequence. Similarly, the vacuolar-sorting sequence of yeast proteinase A is contained within a 16-residue sequence (61–76) near the C terminus of its propeptide (8). These two yeast vacuolar-sorting sequences share the following 6-residue sequence motif (Fig. 7).

Serine 1/spacer 2/spacer 3/positive 4/proline 5/hydrophobe 6

**SEQUENCE 1**
The essential residues for correct sorting of pro-CPY are Glu\textsuperscript{24} to Asn\textsuperscript{56}.
The presence of two positively charged residues at positions 3–5 in the mammalian sequences contrasts with the single positively charged residue at position 4 in the yeast sequences. Also, proline 5 of the yeast sequences is absent from the mammalian sequences. Like the sequence motif that defines the N-terminal signal peptides, this sequence motif is defined in part by sets of phenotypically similar types of amino acids (positive, hydrophobe).

The Lysosomal Proenzyme Receptor—We have shown that binding of procathepsin L to microsomal membranes at pH 5 is mediated by a specific 43-kDa integral membrane protein, or lysosomal proenzyme receptor (LPR), that reacts with the N-terminal portion of the lysosomal enzyme propeptide (Fig. 6) (21). We do not yet know if a single LPR or a family of related LPRs mediates the association of various lysosomal proteases with microsomal membranes at pH 5. If multiple receptors do exist, then some variation in the sequences of the LPR-binding sites would be expected, as seen for the proposed 6-residue membrane-binding sites within the propeptides of the mammalian lysosomal proteases (Fig. 7).

Only proteins that bind to the MPRs or an alternate lysosomal sorting receptor should react with the LPR. Therefore, the proposed 6-residue mammalian sequence motif sequence for binding of lysosomal procathepsins to microsomal membranes at pH 5 would not be physiologically significant if present in a secretory protein or another protein that cannot bind to the MPRs or to an alternate sorting receptor. In order to release the lysosomal protein from the LPR and render the enzyme soluble in the lumen of the lysosome, the binding site should be localized to a peptide removed from the mature enzyme by post-translational proteolysis in the lysosome, but this peptide need not be localized at the N-terminus of the protein. Multiple proteolytic processing steps are characteristic of most lysosomal enzymes, but it has not been clear whether they serve specific functions or merely occur due to the presence of numerous activated proteases in the organelle.

The similarity of the proposed mammalian membrane-binding sequences to the vacuolar-sorting sequences of the two yeast proteases suggests that LPR-mediated membrane binding of lysosomal proteases may be essential for their correct sorting to lysosomes. Mammalian cells may have evolved the MPR-mediated sorting pathway to function in series with the more primitive LPR-mediated pathway, which resembles the vacuolar-sorting pathway used by unicellular eukaryotes. Mutagenesis experiments are in progress to determine whether the essential step in sorting of the lysosomal procathepsins to lysosomes involves binding to the proenzymes to the LPR(s).

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REFERENCES


Addition of Figure 7:

![Figure 7: Alignment of the mouse procathepsin L sequence of peptide B with similar sequences from mammalian procathepsins and vacuolar-sorting sequences from yeast proteases.](image-url)
Lysosomal Proenzyme Receptor