Lysosomal Targeting of Palmitoyl-protein Thioesterase*

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Palmityl-protein thioesterase is a newly described long chain fatty-acid hydrolase that removes fatty acyl groups from modified cysteines in proteins. We have recently identified palmitoyl-protein thioesterase as the defective enzyme in the recessive hereditary neurologic degenerative disorder infantile neuronal ceroid lipofuscinosis (Vesa, J., Hellsten, E., Verkruyse, L. A., Camp, L. A., Rapola, J., Santavuori, P., Hofmann, S. L., and Peltonen, L. (1995) Nature 376, 584–587). A defect in a lysosomal enzyme had been postulated for the disease, but until recently, the relevant defective lysosomal enzyme had not been identified.

In this paper, we present evidence for the lysosomal localization of palmitoyl-protein thioesterase. We show that COS cells take up exogenously supplied palmityl-protein thioesterase intracellularly and that the cellular uptake is blocked by mannose 6-phosphate, a hallmark of lysosomal enzyme trafficking. The enzyme contains endoglycosidase H-sensitive oligosaccharides that contain phosphate groups. Furthermore, palmityl-protein thioesterase cosediments with lysosomal enzyme markers by Percoll density gradient centrifugation. Interestingly, the pH optimum for the enzyme is in the neutral range, a property shared by two other lysosomal enzymes that remove post-translational protein modifications. These findings suggest that palmityl-protein thioesterase is a lysosomal enzyme and that infantile neuronal ceroid lipofuscinosis is properly classified as a lysosomal storage disorder.

Many proteins involved in signal transduction are dependent on covalently attached lipids for membrane localization and function (1). The major classes of lipid modification include N-myristoylation, S-acylation (usually palmitoylation), S-prenylation (farnesylation or geranylgeranylation), and glypia-lylation (farnesylation or geranylgeranylation), and glypia- llylation may be regarded as a potential mechanism for decreasing the signaling activity of αv.

The mechanism for regulated palmitate turnover, including the identification of key enzymes, has yet to be defined. In fact, the eventual fate of any of the protein-bound lipid anchors is unknown. In an initial attempt to study the mechanism of deacylation of Ha-Ras (a farnesylated and palmitoylated protein), we purified and molecularly cloned a thioesterase (palmityl-protein thioesterase (PPT)† that removes palmitate groups from proteins in vitro (8, 9). Although the enzyme was originally purified from the soluble fraction of bovine brain, localization of the enzyme to an extracellular compartment seemed likely because complex asparagine-linked oligosaccharides were found to modify the enzyme and because a cleaved signal peptide was demonstrated at the N terminus of the newly synthesized protein.

We recently identified PPT as the enzyme defective in a neurovisceral storage disorder common in Finland, infantile neuronal ceroid lipofuscinosis (INCL) (10). The disease is characterized by early visual loss and progressive mental deterioration, resulting in a flat electroencephalogram by age 3, with death by 8–11 years of age (11). Marked neuronal loss in the cortex and cerebellum is seen, whereas brainstem neurons are relatively spared. A characteristic finely granular autofluorescent sudanophilic storage material is found throughout the brain and other tissues (12). The material is similar to lipofuscin resulting from normal aging, and INCL has been proposed as a model for accelerated aging in the human brain (13). Biochemical analysis of the storage material has not been particularly revealing, showing a mixture of protein, lipid, and carbohydrate (14). In INCL, saposins A and D appear to be the predominant proteins (14), but the significance of this finding is unclear since saposins accumulate in a number of diverse storage diseases (15). Because a lysosomal enzyme had not been identified as defective in the disease, it has not been classified historically as a lysosomal storage disorder.

In this paper, we demonstrate that PPT is a lysosomal enzyme. Recombinant PPT produced in COS cells is taken up into untransfected cells by a process inhibited by mannose 6-phosphate, and endogenous PPT cosediments with dense lysosomes. These findings provide strong evidence that PPT is indeed a lysosomal enzyme and that INCL is a lysosomal storage disease.

EXPERIMENTAL PROCEDURES

Affinity-purified Polyclonal Antibodies—Three New Zealand White rabbits were each immunized with 300 µg of recombinant bovine brain PPT (9). The antigen was injected intradermally in Freund’s complete adjuvant, and the rabbits were boosted once after a 6-week interval with 150 µg of antigen in Freund’s incomplete adjuvant. An IgG frac-

† The abbreviations used are: PPT, palmitoyl-protein thioesterase; INCL, infantile neuronal ceroid lipofuscinosis; PBS, phosphate-buffered saline; MDBK, Madin-Darby bovine kidney; Endo H, endoglycosidase H; PNGase F, peptide-N-b-D-glucosaminyl)asparagine amidase.
tion was prepared from preimmune and immune sera by protein A-Sepharose CL-4B (Pharmacia Biotech Inc.) chromatography. IgG was affinity-purified by specific binding to a column consisting of bovine PPT coupled to CNBr-activated Sepharose CL-4B (5 mg of protein/ml of gel). Purified IgG was eluted with 0.1 M ammonium hydroxide (pH 10.5), neutralized immediately with glacial acetic acid, and dialyzed overnight at 4°C against 1 liter of borate-buffered saline.

DNA Transfection and Metabolic Labeling—The construction of pCMV-PPT, which contains the full-length bovine PPT coding region in the eukaryotic expression vector pCMV5, was described previously (9). Simian COS-1 cells were maintained in monolayer culture in Dulbecco's modified Eagle's medium (high glucose) supplemented with 10% (v/v) fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B. Cells were plated at a density of 5 × 10^5 cells/ml in 100-mm dishes and incubated for 9 h. The culture medium was then replaced with fresh medium containing 1.5 mM mannose-glucose, and the cells were incubated overnight at 37°C. Two confluent dishes of cells were washed twice with phosphate-buffered saline and once with homogenization buffer (0.25 M sucrose, 1 mM EDTA (pH 7.5)), and each dish was scraped into 1.2 ml of homogenization buffer and passed 20 times through a ball-bearing homogenizer (H and Y Enterprise, Redwood City, CA) with a clearance of 51 μm. The homogenate was centrifuged for 10 min at 400 × g, and the resulting postnuclear supernatant was layered on a discontinuous gradient consisting of a 1.2-mL cushion of 10 × homogenization buffer and 8.5 ml of an 18% Percoll solution in homogenization buffer. The gradient was centrifuged for 60 min at 27,000 rpm (67,000 × g) in a Sorvall T1270 rotor. The supernatant was collected; the cells were pelleted using a centrifuge, and the Percoll was removed by centrifugation for 40 min at 70,000 rpm in a Bedman TL 100.3 rotor. The samples were adjusted to 0.5% Triton X-100, passed five times through a 25-gauge needle, and incubated on ice for 30 min. β-Hexosaminidase activity (lysosomal marker) was determined as described (18). Assays for enzymes other than β-hexosaminidase were performed exactly as described (19). For determination of the location of endosomes, the endosomal compartment was labeled with horseradish peroxidase in a parallel experiment by incubation of cells for 5 min at 37°C in medium containing 10 mg/ml horseradish peroxidase, processed, and assayed as described (19).

Oligosaccharide Analysis—COS-1 cells in 60-mm dishes were transfected with pCMV-PPT, and at 48 h post-transfection, the cells were washed three times with phosphate-buffered saline and incubated in 0.8 ml of Dulbecco's modified Eagle's medium containing 0.5 mM glucose, 2 mM L-glutamine, 10% dialyzed bovine serum, 12.5 mM ammonium chloride (to promote secretion of lysosomal enzymes), and 200 μCi of [3H]mannose (n-[2-3H]mannose, American Radiolabeled Chemicals) for 4 h at 37°C. Normal maintenance medium was added to the dishes, and the medium was adjusted to 5 mM glucose and 5 mM mannose to inhibit further uptake of [3H]mannose. After an additional 4 h, the medium was harvested and centrifuged at 4°C against 500 ml of a buffer containing 25 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 0.2% sodium azide at 10,000 rpm for 30 min. The dialysate was collected and then was subjected to electrophoresis using anti-bovine PPT antibodies, and the [3H]mannose-labeled immune complexes were analyzed by endo H or N-glycanase treatment by QAE-Sephadex chromatography (for determination of the degree of phosphorylation) and concanavalin A chromatography (for determination of high mannose and b, tri-, and tetraantennary complex oligosaccharides) exactly as described (20).

RESULTS

The synthesis and secretion of bovine recombinant PPT were examined in a transient transfection assay using simian COS cells that were pulse-labeled with [35S]cysteine/methionine and chased for varying periods of time using unlabeled medium (Fig. 1). [35S]-Labeled PPT was immunoprecipitated and analyzed by SDS-sodium dodecyl sulfate gel electrophoresis and fluorography either before (Fig. 1, upper panels) or after (lower panels) treatment with Endo H, a treatment that releases high-mannose-type, but not complex, oligosaccharides from the polypeptide backbone. Newly synthesized PPT appeared as a doublet at 38.5 and 36 kDa (Fig. 1, first lane). Labeled enzyme appeared in the medium by 30 min of chase, and by 1 h of chase, half of the label had appeared in the culture medium, with a peak for the appearance of labeled PPT in the medium at 3 h. All of the labeled enzyme associated with the cells was sensitive to Endo H treatment (Fig. 1, lower left panel), while the
medium contained a mixture of Endo H-sensitive and Endo H-resistant enzyme (Fig. 1, lower right panel), with a predominance of the Endo H-resistant form.

Many proteins normally targeted to intracellular compartments are secreted into the culture medium when overexpressed in COS or other transfected cells. To determine whether PPT is destined for secretion or whether it might be targeted to an intracellular organelle, we added culture medium from PPT-overexpressing COS cells to untransfected COS cells and found that PPT was taken up and internalized by the untransfected cells (Fig. 2, lane 1). Furthermore, cellular uptake was inhibited by mannos 6-phosphate (Fig. 2, lanes 5–7), but not by mannose alone (lanes 2–4). This result is diagnostic of uptake through mannose 6-phosphate receptor-mediated endocytosis, a distinguishing feature of lysosomal enzyme transport (21).

To further support this observation, an experiment was performed to demonstrate that phosphorylated high-mannose oligosaccharides are bound to PPT (Fig. 3). COS cells were transiently transfected with bovine recombinant PPT and incubated in the presence of $^{32}$P-labeled orthophosphate. Cell lysates and culture medium were subjected to immunoprecipitation and analysis on SDS-polyacrylamide slab gels and autoradiography. In the presence of immune serum, a clear $^{32}$P-labeled band was seen in the cells (Fig. 3, lane 2) and culture medium (lane 5). The $^{32}$P label was removed completely following incubation of the immunoprecipitates with endoglycosidase H, which removes high-mannose oligosaccharide chains, thus confirming the presence of the mannose 6-phosphate modification of PPT.

A quantitative oligosaccharide analysis of bovine recombinant PPT immunoprecipitated from COS cell-conditioned medium (Table I) was undertaken to further characterize these modifications. The transfected cells were cultured in the presence of ammonium chloride, which promotes the secretion of lysosomal enzymes (18). We found that 30.4 ± 1.1% of the total oligosaccharide chains were monophosphorylated, and 21.1% were diphosphorylated (all of the high-mannose type); furthermore, 44.6 ± 1.8% of the oligosaccharides were of the complex type. The majority of these complex oligosaccharides were of the biantennary form.

The lysosomal localization of endogenous PPT in MDBK cells was confirmed by Percoll density gradient centrifugation (Fig. 4). MDBK cells growing in monolayer culture were disrupted using a ball-bearing homogenizer and loaded onto a self-forming Percoll gradient. Following centrifugation, fractions were collected for marker enzyme analysis and immunoblotting with anti-bovine PPT antibodies. Using this technique, dense lysosomes pelleted in the bottom fraction, whereas mitochondrial, endocytic, Golgi, and plasma membrane markers were included in the gradient (Fig. 4, upper panel). PPT immunoreactivity was essentially entirely restricted to the bottom fraction, a

**FIG. 2. Inhibition of uptake of recombinant bovine PPT into COS cells by mannose 6-phosphate.** COS cell-conditioned medium containing recombinant bovine PPT was incubated overnight with untransfected cells. Cell lysates were subjected to electrophoresis on a 12% SDS-polyacrylamide gel and transferred to nitrocellulose for immunoblotting using anti-bovine PPT antibodies. No signal is seen using preimmune serum (data not shown). The doublet at 66 kDa is a cross-reacting band seen in untransfected COS cells. Shown is one of two experiments giving similar results.

**FIG. 3. Incorporation of $^{32}$P into recombinant PPT and sensitivity to Endo H.** COS cells transfected with pCMV-PPT were labeled with $^{32}$P orthophosphate for 3 h. Cell lysates and medium were subjected to immunoprecipitation with either preimmune (PI) or immune (IM) anti-bovine PPT antibodies, and the immunoprecipitates were incubated in the presence or absence of Endo H and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Exposure was for 3 days at −85 °C using an intensifying screen.

**FIG. 1. $^{35}$S Cysteine/methionine pulse-chase labeling of recombinant bovine palmitoyl-protein thioesterase in transiently transfected simian COS cells.** Cells were transfected with plasmid pCMV-PPT, pulse-labeled for 15 min, and chased in the presence of unlabeled medium for varying periods of time as indicated. The culture medium and cells were collected, and lysates were subjected to immunoprecipitation with or without incubation with Endo H as indicated, followed by electrophoresis on 12% SDS-polyacrylamide slab gels and fluorography for 9 h at −85°C. No specific signal was seen using cells transfected with the vector alone or with preimmune serum (data not shown). Data shown represent one of three independent experiments giving similar results.

**TABLE I**

| Oligosaccharide Analysis of Bovine Recombinant PPT from COS Cell-Conditioned Medium | % of Total (Mean ± S.E.) |
|-----------------------------|-------------------------|
| Endo H-sensitive oligosaccharides | 3.9 ± 1.2 |
| Unphosphorylated | 30.4 ± 1.1 |
| Monophosphorylated | 21.2 ± 2.1 |
| Diphosphorylated | 12.0 ± 3.6 |
| Endo H-resistant oligosaccharides | 44.6 ± 1.8 |
| Tri- and tetraantennary complexes | 51.5 ± 2.0 |

The lysosomal localization of endogenous PPT in MDBK cells was confirmed by Percoll density gradient centrifugation (Fig. 4). MDBK cells growing in monolayer culture were disrupted using a ball-bearing homogenizer and loaded onto a self-forming Percoll gradient. Following centrifugation, fractions were collected for marker enzyme analysis and immunoblotting with anti-bovine PPT antibodies. Using this technique, dense lysosomes pelleted in the bottom fraction, whereas mitochondrial, endocytic, Golgi, and plasma membrane markers were included in the gradient (Fig. 4, upper panel). PPT immunoreactivity was essentially entirely restricted to the bottom fraction, a
and all of these are utilized to some degree. 2) PPT recovered forms. (PPT has three potential sites, with only a subset subjected to phosphorylation and expected in the case of a protein that contains multiple glycosylation sites. This result would be expected as a lysosomal localization (Fig. 4, lower panel). The fractions were also analyzed for PPT enzymatic activity using [3H]palmitate-labeled Ha-Ras as a substrate (data not shown). The vast majority of the activity correlated exactly with PPT immunoreactivity, while a minor activity peaked with the plasma membrane marker and probably represents an N-ethylmaleimide- and phenylmethanesulfonyl fluoride-sensitive membrane-bound thioesterase described previously (8)). This activity is currently being characterized further.

A study of the biosynthesis of endogenous PPT in MDBK cells was not technically feasible owing to the low abundance of the protein. However, an examination of the migration pattern of PPT by immunoblotting of cell lysates and conditioned medium from the MDBK cells (Fig. 5) revealed evidence for processing of the oligosaccharide chains from high mannose-type to complex oligosaccharides during the maturation of the endogenous enzyme. Digestion of cell-associated PPT with Endo H caused a large shift of PPT immunoreactive bands (Fig. 5, compare lanes 1 and 3), indicating that most of the cell-associated enzyme contains high-mannose oligosaccharides. However, very little enzyme was completely deglycosylated by this treatment (compare lanes 3 and 2, which was PNGase F-treated), indicating that most molecules contain both high-mannose and complex oligosaccharide chains. This result would be expected in the case of a protein that contains multiple glycosylation sites, with only a subset subjected to phosphorylation and the remainder being processed to complex Endo H-resistant forms. (PPT has three potential N-linked glycosylation sites, and all of these are utilized to some degree.) PPT recovered from the medium migrated at an even higher molecular mass, as expected for a lysosomal enzyme that has escaped mannose 6-phosphate receptor uptake and has been processed to complex forms (Fig. 5, lane 4). Consistent with this prediction is the result shown in Fig. 5 (lane 6), in which a larger proportion of the secreted enzyme appears to be Endo H-resistant as compared with the cell-associated enzyme.

Many lysosomal enzymes undergo extensive proteolytic processing during maturation (reviewed in Ref. 22). However, we did not find evidence for significant proteolytic processing of PPT during synthesis or reuptake. This is best seen by a comparison of the 35S-labeled bands during the pulse and chase phases of biosynthetic labeling (Fig. 1) and by a comparison of the migration of cellular, secreted, and endocytosed forms of recombinant PPT in COS cells (Fig. 6, lanes 1–3), particularly following deglycosylation with Endo H or PNGase F (lanes 4–9). No shift in the migration of deglycosylated PPT was seen either after secretion or after endocytosis (Fig. 6, lanes 4–6 and 7–9). We cannot rule out a small amount of proteolytic processing occurring at either end of the mature protein; in fact, PPT purified from brain has two fewer N-terminal amino acids as compared with recombinant bovine PPT expressed in the insect cell-baculovirus expression system (see "Discussion"). This minor form of processing would not have been detected in these experiments. The Sf9 cell-derived enzyme is not phosphorylated on mannose residues and is not competent for uptake through the mannose 6-phosphate receptor (data not shown).

In addition to proteolytic processing in the lysosome, lysosomal enzymes often lose some of their carbohydrate by the action of lysosomal exoglycosidases (23). However, the trimming of terminal carbohydrate residues is not necessary for the function of these enzymes, and the significance of these alterations is unknown. The shift in migration seen when the secreted form of PPT is taken up by the untransfected cells (Fig. 6, compare lanes 2 and 3) may be attributed to this type of processing; alternatively, it is also possible that only a small fraction of enzyme molecules bearing mannose 6-phosphate residues are subject to endocytosis, and the apparent shift results from the selection of only this fraction, which may migrate at a lower apparent molecular mass. A more sophisticated analysis of the oligosaccharides bound to PPT at various stages of maturation will be needed to distinguish between these possibilities.

We had previously reported (8) that the pH optimum of PPT using Ha-Ras as a substrate is around 7.0, an unusual finding for lysosomal enzymes, which usually have pH optima in the acidic range. We have confirmed and extended this observation in thioesterase assays using both [3H]palmitate-labeled Ha-Ras and [3H]palmitoyl-CoA as substrates (Fig. 7). The pH optimum using Ha-Ras as a substrate is broad, but clearly peaks at pH 7.0 (Fig. 7A). However, significant residual activity was seen at lower pH values (10–20% at pH 4.5 and ~25–50% of maximal at pH 5.5, with some variations due to buffer effects). The pH optimum using the model substrate palmitoyl-CoA (Fig. 7B) is pH 7.5, but activity was half-maximal in the range 4.5–5.5.

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2 J.-Y. Lu and S. L. Hofmann, unpublished results.
confirmed by Percoll density gradient ultracentrifugation, in targeting. The localization of PPT to lysosomes was further alone, which is a distinguishing feature of lysosomal enzyme manner blocked by mannose 6-phosphate, but not by mannose H-sensitive phosphate and to be taken up by COS cells in a nant PPT expressed in COS cells was found to contain Endo H-sensitive phosphatase and to be taken up by COS cells in a

In this study, PPT was shown to undergo both early and late processing events characteristic of lysosomal enzymes. We know that the signal peptide is removed by comparison of the deduced amino acid sequence of PPT with the N-terminal se- quence of purified brain and recombinant forms of the enzyme. In this study, evidence for the modification of oligosaccharides by mannose 6-phosphate and further processing to complex and hybrid forms is presented. We saw little evidence for proteolytic fragmentation of PPT; however, we know that two amino acids are removed from the N terminus in a late processing event because the mature form purified from bovine brain begins at residue 26, and the recombinant form derived from the Sf9 insect cell-baculovirus system begins at residue 26. We have also previously shown that the Sf9 form is fully active, and this minor proteolytic trimming does not serve to activate the protein.

In many respects, PPT is synthesized and processed like a “typical” lysosomal enzyme. However, the neutral pH optimum we observed for the enzyme is puzzling. Why should a lysoso- mal enzyme have a neutral pH optimum? We are aware of only two other lysosomal enzymes (aspartylglucosaminidase (25), the enzyme defective in aspartylglucosaminuria, another “Finnish disease heritage” enzyme, and a lysosomal sialic acid-specific O-acetylerase (26)). Interestingly, all three of these enzymes have been postulated to have a role in the removal of post-translational protein modifications in the lysosome. Butor et al. (27) have recently presented evidence for the intermittent cycling of lysosomes to more neutral pH. These investigators have put forth the interesting idea that periodic pH fluctua- tions would promote protein denaturation, allowing access of degradative enzymes to the modified amino acid residues. Such an explanation is attractive in the case of PPT, which recog-

**FIG. 6. Processing of oligosaccharides accounts for migration differences on SDS-polyacrylamide gels of various recombinant PPT forms.** C, whole cell extract; S, culture medium (secreted form); E, endocytosed form of PPT. PPT is present as a single band at 31 kDa following PNGase F or Endo H digestion. The Endo H-treated form comigrates with the PNGase F-treated form when the two forms are run in adjacent lanes (data not shown). A small amount of Endo H-resistant PPT is found in the culture medium (lane 8).

**DISCUSSION**

In this paper, we provide evidence for the lysosomal localization and targeting of palmitoyl-protein thioesterase. Recombi- nant PPT expressed in COS cells was found to contain Endo H-sensitive phosphate and to be taken up by COS cells in a manner blocked by mannose 6-phosphate, but not by mannose alone, which is a distinguishing feature of lysosomal enzyme targeting. The localization of PPT to lysosomes was further confirmed by Percoll density gradient ultracentrifugation, in which all of the PPT in MDBK cells was found to cosediment with dense lysosomes.

Lysosomal enzymes undergo a number of modifications during synthesis and processing on their way to the lysosomal compartment. These modifications are classified as early (pre-lysosomal) or late (post-lysosomal) processing events (reviewed in Ref. 22). Soluble lysosomal enzymes are synthesized in the rough endoplasmic reticulum. The nascent polypeptides are cotranslationally separated from signal sequences and then glycosylated in the lumen of the rough endoplasmic reticulum. The early oligosaccharides are of the high-mannose type. In the endoplasmic reticulum and in the Golgi apparatus, the oligo- saccharides of lysosomal proteins are subjected to further proc- essing. In the cis-Golgi, a signal “patch” on the lysosomal en- zyme directs the addition of an N-acetylgalosaminyl phosphate to the C-6 hydroxy groups of one or two mannose residues of oligosaccharides at particular glycosylation sites (24). If this phosphorylation is not allowed to proceed (as in the case of overexpression of recombinant proteins, in which pro- tein production may exceed the capacity of phosphoryl trans- fer), the oligosaccharides may be further processed and come to contain predominately complex or hybrid forms that are resistant to Endo H. Unphosphorylated enzymes are secreted, as are a small proportion of phosphorylated enzymes that escape capture by the mannose 6-phosphate receptor. These events are referred to as early forms of lysosomal enzyme processing (22). Lysosomal enzyme precursors that are targeted to lysosomes are also subjected to late processing, which may consist of fragmentation of the protein (in rare cases, leading to activation of a latent form of the enzyme), trimming of the oligosac- charide side chains, or both. In a few instances, the fragmenta- tion process involves activation of a zymogen, as is the case for several of the cathepsins (D, L, H, and B). Little if any N-terminal processing (beyond removal of the signal sequence) is seen in a number of other human lysosomal enzymes (22).

In this study, PPT was shown to undergo both early and late processing events characteristic of lysosomal enzymes. We know that the signal peptide is removed by comparison of the deduced amino acid sequence of PPT with the N-terminal se- quence of purified brain and recombinant forms of the enzyme. In this study, evidence for the modification of oligosaccharides by mannose 6-phosphate and further processing to complex and hybrid forms is presented. We saw little evidence for proteolytic fragmentation of PPT; however, we know that two amino acids are removed from the N terminus in a late processing event because the mature form purified from bovine brain begins at residue 26, and the recombinant form derived from the Sf9 insect cell-baculovirus system begins at residue 26. We have also previously shown that the Sf9 form is fully active, and this minor proteolytic trimming does not serve to activate the protein.

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nizes palmitate residues that modify cysteines in proteins, and in the case of the sialic-acid esterase, which removes sialic acid residues from modified proteins. However, aspartylglucosaminidase recognizes the modified asparagine only after it has been liberated from the protein backbone, and so would not be aided in its action by protein denaturation.

Defective PPT function leads to the storage disorder infantile neuronal ceroid lipofuscinosis (10). The disease is characterized by the accumulation of membrane-delimited intracellular inclusion bodies that stain with antibodies directed against acid hydrolases by immunohistochemistry (28). Therefore, the demonstration that PPT is a lysosomal enzyme conclusively supports the classification of INCL as a lysosomal storage disease. Therapeutic strategies designed to target defective lysosomal enzymes to the central nervous system (23) should therefore be relevant to INCL.

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