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Posttranslational Processing of Human Lysosomal Acid β-Glucosidase: A Continuum of Defects in Gaucher Disease Type 1 and Type 2 Fibroblasts

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Summary

The major processing steps in the maturation of the lysosomal hydrolase, acid β-glucosidase, were examined in fibroblasts from normal individuals and from patients with types 1 and 2 Gaucher disease. In pulse-chase studies with normal fibroblasts, remodeling of N-linked oligosaccharides resulted in the temporal appearance of three molecular-weight forms of acid β-glucosidase. An initial 64-kDa form, containing high mannose-type oligosaccharide side chains, was processed quantitatively, within 24 h, to a sialylated 69-kDa form. During the subsequent 96 h, some of the 69-kDa form is processed to 59 kDa. Glycosidase digestion studies revealed that the increase in the apparent molecular weight of the normal enzyme from 64 kDa to 69 kDa resulted primarily from the addition to sialic acid residues in the Golgi apparatus. The polypeptide backbone of both the 64-kDa and 69-kDa forms was 55.3 kDa. Processing of acid β-glucosidase in fibroblasts from three of four type 1 (nonneuronopathic) Ashkenazi Jewish Gaucher disease patients was nearly normal. With fibroblasts from one Ashkenazi Jewish and three non-Jewish type 1 as well as from two type 2 (acute neuronopathic) Gaucher disease patients, only a 64-kDa form of acid β-glucosidase was detected. Inefficient and incomplete processing to the 69-kDa form was found in one type 2 cell line (GM2627). These results indicate that no firm correlation exists between the type or degree of abnormal processing of acid β-glucosidase in fibroblasts and the phenotype of Gaucher disease.

Introduction

Human acid β-glucosidase (N-acyl-sphingosyl-O-β-D-glycoside: glucohydrolase [E.C.3.2.1.45]) is a membrane-associated lysosomal glycoprotein (Mueller and Rosenberg 1977) which cleaves the β-glucosidic linkage of glucosylceramide and synthetic β-glucosides (Brady et al. 1965). The molecular mass of deglycosylated mature acid β-glucosidase subunit (55,834 Da) and its preenzyme(s) forms (57.5 and 59.8 kDa) have been calculated from the polypeptides predicted from the nucleotide sequence of the cDNA (Sorge et al. 1985; Tsuji et al. 1986). The mature porcine enzyme has oligosaccharides on four of its five possible N-glycosylation sites (Erickson et al. 1985). Inherited defects in this enzymatic activity are etiologic for the phenotypes of Gaucher disease (Brady et al. 1965). The resultant progressive accumulation of glucosylceramide and glucosylsphingosine, primarily within lysosomes of monocyte/macrophage-derived cells (Frederickson and Sloan 1972; Parkin and Brunning 1982), leads to the clinical manifestations of Gaucher disease. Three major phenotypes have been delineated, based on the absence (type 1) or presence (and severity [types 2 and 3]) of neuronopathic involvement (Frederickson and Sloan 1972; Desnick 1982). The lack of complementation of fibroblasts from types 1, 2, and 3 patients with Gaucher disease indicated that these phenotypes result from allelic mutations in a common gene (Gravel and Leung 1983; Wenger et al. 1983).

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In vitro kinetic (Grabowski et al. 1985*a*, 1985*b*) and immunoblotting pattern (Ginns et al. 1982; Fabbro et al. 1987) differences of the residual enzyme have been demonstrated within and among the disease phenotypes. These findings and the presence of normal amounts and quality of acid β-glucosidase mRNAs (Graves et al. 1986) in several Gaucher disease variants suggested an altered processing (and/or stability) of the mutant enzymes. On the basis of such findings, investigators speculated that the causal mutations in the nonneuronopathic (type 1) and neuronopathic (types 2 and 3) phenotypes of Gaucher disease resulted in differentially altered processing of acid β-glucosidases (Ginns et al. 1982).

Here we report on temporal studies of the processing of acid β-glucosidase from cultured fibroblasts of normal individuals and types 1 and 2 Gaucher disease patients. The results provided evidence for extensive remodeling of the N-linked oligosaccharide chains of the normal enzyme after leader peptide cleavage, but no posttranslational processing of the polypeptide chain itself. The studies of the mutant enzyme from Gaucher disease patients demonstrated normal, inefficient, or absent processing of mutant enzymes in both types 1 and 2 Gaucher disease fibroblasts. These results indicated that the allelic mutations which result in the subtypes and variants of Gaucher disease can lead to similar posttranslational glycosidic processing alterations in fibroblasts which are not characteristic for a particular phenotype.

Material and Methods

Material

The following were obtained from commercial sources: FBS and RPMI 1640 (Gibco, Grand Island, NY); Triton X-100, sodium deoxycholate, and phenylmethylsulfonyl fluoride (PMSF) (Sigma, St. Louis); endoglycosidase H (endo H) (Boehringer Mannheim Biochemicals, Indianapolis); neuraminidase (Calbiochem, San Diego); N-Glycanase™ (Genzyme, Boston); fixed *Staphylococcus aureus* cells (BRL, Bethesda, MD); and ^{35}S-methionine (ICN, Cleveland).

Methods

Fibroblast sources, cultures, and ^{35}S-methionine labeling.—Fibroblast cultures were established from skin explants by standard procedures, after informed consent had been obtained according to institutional and NIH guidelines. Assent was obtained from children under the age of 16 years. Cell lines GM877, GM1260, and GM2627 were obtained from the NIGMS Human Genetic Mutant Cell Repository (Camden, NJ). Cell lines from four type 1 Ashkenazi Jewish and three type 1 non-Jewish Gaucher disease patients were also studied. None of the patients whose cells were used in these studies were consanguineous.

Skin fibroblast cultures were grown under RPMI 1640 supplemented with 12% FBS. The passage number for all experiments was 5–15. For each experiment, cells were plated on 50-mm tissue culture dishes at a confluence of 10%–50%. The cells were incubated at 37°C under 5% CO_2. The medium was changed every 2 d until the cells had reached confluency. Prior to being labeled, the confluent cells were washed twice with sterile PBS (150 mM NaCl, 25 mM Na_2HPO_4, 25 mM K_2HPO_4 pH 7.4). Prewarmed (37°C) labeling medium (2 ml Eagle's minimal essential medium lacking methionine [Flow Laboratories, McLean, VA]) supplemented with 10% dialyzed FBS and 50 μCi/ml ^{35}S-methionine was added immediately following the washes. After incubation for 30 min at 37°C, the labeling medium was removed and replaced with prewarmed (37°C) growth medium. The cells then were incubated at 37°C for the indicated times (chase period). After the designated chase period, the cells were harvested by lysis in 1 ml PBS containing 1% Triton X-100, 0.5% sodium deoxycholate, and 0.2 mM PMSF. The nuclei were removed by centrifugation in an Eppendorf Microfuge™ (model 5414) for 2 min. The lysate supernatants were quick frozen and stored under liquid nitrogen until all the time points had been obtained.

Immunoprecipitation.—The lysate supernatants for each time point were thawed and adjusted to 0.3% SDS. Then, 50 μl of a 10% suspension of fixed washed *Staphylococcus aureus* cells were added to each tube, incubated for 15 min (0°C), and removed by centrifugation for 2 min in an Eppendorf Microfuge™ (model 5414). Rabbit anti-human acid β-glucosidase IgG (Fabbro et al. 1987) was precleared of aggregates by centrifugation at 30,000 *g* for 30 min in a Sorvall SS-34 rotor (Dupont), and 1 μl was added to each lysate supernatant. Pilot experiments indicated that this quantity of specific IgG quantitatively removed acid β-glucosidase activity from comparable cultures of normal human fibroblasts. The lysate-IgG mixtures were incubated overnight at 0°C, and any aggregates in these mixtures were cleared by centrifugation at 30,000 *g* for 30 min (4°C). This step significantly reduces the nonspecific background. To ensure that there was no loss of acid β-glucosidase, the activity in the normal

cell extracts was tested before and after this centrifugation step (the antibody used in these studies does not inhibit enzyme activity). No activity was lost during the centrifugation. In addition, no activity was detected in the pellet.

After being cleared, the supernatants were transferred into tubes containing 50 μl washed fixed *S. aureus* cells and were incubated for an additional 15 min (0°C). The immune complexes were harvested by centrifugation for 1 min in an Eppendorf Microfuge™ and were washed five times in 10 mM Tris-HCl (pH 7.8) containing, 10 mM EDTA, 1% Triton X-100, 0.4% sodium deoxycholate, and 0.1% SDS. The detergents were removed from the immune complexes by a final wash in 10 mM Tris-HCl at pH 7.8. When the samples were to be immediately subjected to gel electrophoresis, the washed precipitates were disaggregated by heating (100° C for 5 min) in electrophoresis sample buffer (62 mM Tris-HCl pH 6.8, containing 2% SDS, 20% glycerol, 140 mM beta-mercaptoethanol, and 0.002% bromophenol blue).

Glycosidase digestions.—Neuraminidase digestions were carried out as described by Rogalski et al. (1984). After digestion, the precipitates (still associated with *S. aureus* cells) were collected by centrifugation for 2 min in an Eppendorf Microfuge™. They were disaggregated in 50 μl electrophoresis sample buffer by being heated to 100°C for 5 min, and the *S. aureus* cells were removed by centrifugation in a microfuge for 2 min.

For endo H digestions, the immunoprecipitated protein was disassociated from the *S. aureus* cells by being heated to 100°C in 30 μl 15 mM DTT, 50 mM Tris-HCl pH 6.8, and 1% SDS for 5 min. The *S. aureus* cells were then removed by centrifugation in a microfuge for 2 min. Each reaction contained 30 μl 200 mM sodium citrate pH 5.5, 10 μl disassociated sample, and 10 μl endo H (0.05 units/ml in 42 mM citric acid, 104 mM sodium phosphate dibasic pH 5.5). Samples were digested overnight at 37°C.

For N-Glycanase™ digestions, the immunoprecipitated protein was disassociated from the *S. aureus* cells by being heated to 100°C in 50 μl 0.5% SDS, 100 mM beta-mercaptoethanol for 5 min. The *S. aureus* cells were then removed by centrifugation in an Eppendorf Microfuge™ for 2 min. Each reaction contained 10 μl SDS glycoprotein solution, 8.8 μl 0.68 M sodium phosphate pH 8.6, 5 μl 30 mM EDTA, 5 μl 7.5% NP40), and 0.3 units N-Glycanase.™ Samples were digested overnight at 37°C. After endo H and N-Glycanase™ digestions, 20 μg bovine hemoglobin (Sigma) and 600 μl acetone were added to each sample. Precipitates were allowed to form for 15 min at 0°C. The precipitates were collected by centrifugation for 10 min in an Eppendorf Microfuge™. After drying, the precipitates were resuspended in 50 μl electrophoresis sample buffer.

Gel electrophoresis.—All samples were subjected to electrophoresis in 10% polyacrylamide gels according to the method described by Laemmli (1970). After electrophoresis, the gels were fixed, stained, destained, washed in H_2O, impregnated with 1 M sodium salicylate, and dried, ^{35}S-methionine labeled proteins were visualized by fluorography. Each figure indicates the positions of radiolabeled vesicular stomatitis virus protein standards. The molecular weights of the L (241 kDa), N (47 kDa), and M (26 kDa) proteins were calculated from their complete amino acid sequences (Gallione 1981; Rose and Gallione 1981; Schubert et al. 1984). Owing to the extensive posttranslational modification of the G protein, its predicted molecular weight was not used. The value used, 65 kDa, represents the consensus of molecular-weight determinations from the literature (Mudd and Summers 1970; Wunner and Pringle 1972; Obijeski et al. 1974). The molecular weights of the three forms of β-glucosidase were determined from both their migration and a linear-regression best-fit analysis of the migration of the standards versus the logarithm of the standards' molecular weights.

Results

Processing of Acid β-Glucosidase in Fibroblasts from Normal Individuals

The time course for processing of acid β-glucosidase from normal fibroblasts is shown in figure 1. The molecular weight of each of the three enzyme forms was determined as described in the Methods section. After a 30-min pulse with ^{35}S-methionine, an acid β-glucosidase form with an apparent molecular weight of 64 kDa was observed. Subsequently, this form was processed to a form with an apparent molecular weight of 69 kDa. The half-time for this processing was approximately 3 h. After an additional 48–96 h, a third form of acid β-glucosidase, with apparent molecular weight of 59 kDa, was observed.

Since the 64-kDa form of the normal enzyme was the first species detected after a 30-min labeling period, it was likely to contain high-mannose-type oligosaccharides. Furthermore, the half-time for its conversion to the 69-kDa molecular-weight form suggested that the apparent change in molecular weight might be due to maturation of these oligosaccharides, to complex

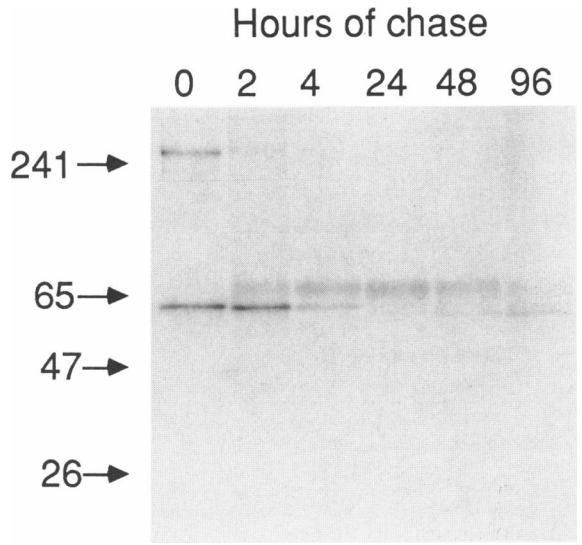

Figure 1 Time course of processing of acid β-glucosidase in normal human fibroblasts. Cell line 4467 fibroblasts were labeled for 30 min with ^{35}S-methionine and were chased for the times indicated above each lane.

type, in the Golgi apparatus. To directly test these possibilities, the sensitivity of the oligosaccharides on these forms of the normal acid β-glucosidase to treatment with endo H and neuraminidase was evaluated. High-mannose oligosaccharides are known to be cleaved by endo H, resulting in a change in the apparent molecular weight of their parent protein. In contrast, the complex-type oligosaccharides arising from remodeling in the Golgi apparatus are completely resistant to endo H (Maley and Trimble 1981).

Figure 2*A* shows the mobility of normal acid β-glucosidase before and after endo H treatment. The oligosaccharides on the 64-kDa form of acid β-glucosidase were sensitive to endo H digestion: i.e., after digestion, the 64-kDa form of the enzyme migrated with an apparent molecular weight of 55.3 kDa (fig. 2*A*). This molecular weight is in excellent agreement with that for the mature deglycosylated polypeptide (55.8 kDa) predicted from the cloned cDNA (Sorge et al. 1985; Tsuji et al. 1986). In comparison, the 69-kDa form of the enzyme was substantially resistant to digestion with endo H. After treatment, it shifted to an apparent molecular weight of 66 kDa. This shift in mobility is consistent with the loss of one or possibly two N-linked high-mannose-type oligosaccharides. It is likely, therefore, that all but one of the asparagine-linked oligosaccharides of human β-glucosidase are processed to complex-type forms in the Golgi apparatus. To test whether this high-molecular-weight form of acid β-glucosidase contained complex-type oligosaccharides with terminal sialic acid residues, the mobility of the 69-kDa form was compared before and after treatment with neuraminidase. As shown in figure 2*C*, the higher-molecular-weight form was converted into a faster-migrating species, with an apparent molecular weight of 65 kDa, by neuraminidase treatment. The mobility of the 64-kDa form was not affected by neuraminidase treatment. These results indicate that the 69-kDA form arises by the addition of terminal sialic acid residues to the complex-type oligosaccharides on acid β-glucosidase in the

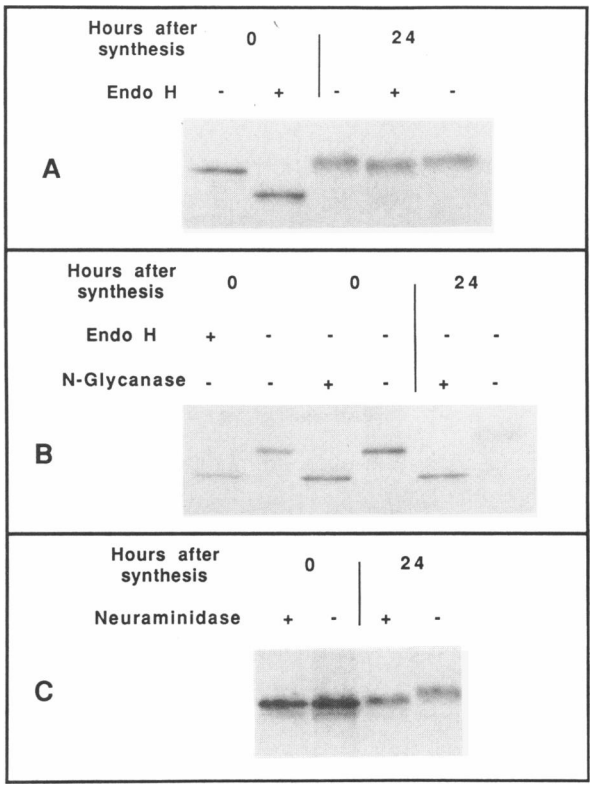

Figure 2 Glycosidase sensitivities of different forms of normal acid β-glucosidase. Normal skin fibroblast cell line 4467 was labeled with ^{35}S-methionine for 30 min and then either was harvested immediately or was incubated in normal growth medium for 24 h. Acid β-glucosidase was isolated by immunoprecipitation and subjected to digestion with either endoglycosidase H (Endo H) (panels *A* and *B*), N-Glycanase™ (panel *B*), or neuraminidase (panel *C*).

Golgi apparatus. To determine whether the shift from 64 kDa to 69 kDa might be accompanied by proteolytic processing, both these forms of normal acid β-glucosidase were treated with N-Glycanase™, an enzyme that removes all N-linked oligosaccharides from proteins (fig. 2*B*). After treatment with N-Glycanase™, these forms of acid β-glucosidase had the same apparent molecular weight (55.3 kDa). The resolution of the SDS-polyacrylamide gels achieved in these studies were sufficient to detect a molecular weight change of 1–2 kDa. Thus, fewer than 20 amino acids could have been removed from acid β-glucosidase during the 24 h after its synthesis.

Processing of Acid β-Glucosidase in Fibroblasts from Patients with Type 1 Gaucher Disease

Results of processing studies of acid β-glucosidase in fibroblasts from a representative patient (AW) with the Ashkenazi Jewish variant of type 1 (nonneuronopathic) Gaucher disease are shown in figure 3. The results in figure 3 were obtained on an 11-year-old Ashkenazi Jewish patient with type 1 disease. All three normal forms of acid β-glucosidase were detected. However, the rate of conversion of acid β-glucosidase to the 69-kDa form was less efficient than in cells from normal individuals. In comparison with the normal cells, the acid β-glucosidase in these fibroblasts failed to be quantitatively converted from the 64-kDa form to the 69-kDa form (compare figs. 1, 3). The final conversion to the 59-kDa form proceeded at an apparently normal rate. This processing pattern was observed in fibroblasts from three of the four type 1 Ashkenazi Jewish Gaucher disease patients studied.

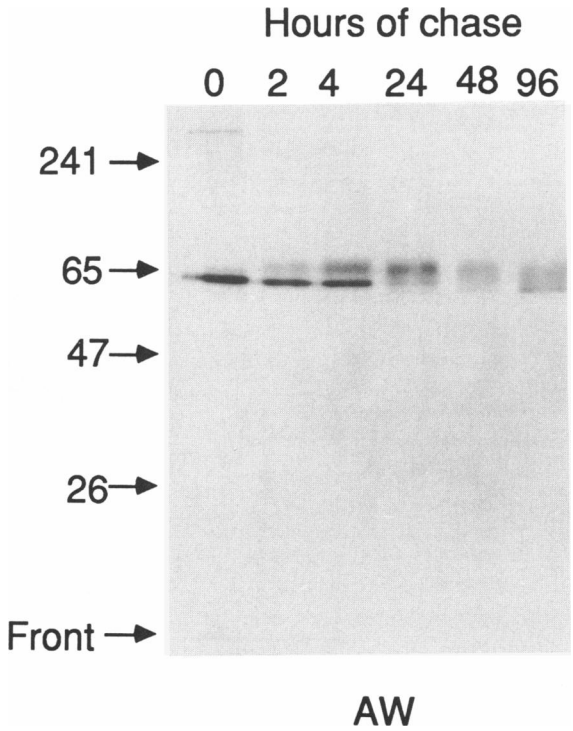

Figure 3 Time course of processing of acid β-glucosidase in typical Ashkenazi Jewish type 1 Gaucher disease fibroblasts. Cell line AW fibroblasts were labeled for 30 min with ^{35}S-methionine and were chased for the times indicated above each lane.

The processing of acid β-glucosidase in fibroblasts from other patients with type 1 Gaucher disease differed from that in normal fibroblasts. The results in figures 4 and 5 were those obtained from patients B303 and B389. These type 1 Gaucher disease patients were 9 and 11 years old, respectively. B303 was of Ashkenazi Jewish lineage, and B389 was of non-Jewish Caucasian descent. It is important to note that the phenotype of patient AW, whose results are shown in figure 3, and those of B303 and B389 were indistinguishable.

Figures 4 and 5 show the time courses of acid β-glucosidase modification by fibroblasts from B303 and B389, respectively. In both cases, the 64-kDa form of acid β-glucosidase was synthesized, and little, if any, processing was observed. A very faint band at 69 kDa was observed, but no increase in intensity of this form was observed after 2–4 h of chase. In addition, the 64-kDa form rapidly disappeared. This processing pattern was observed in fibroblasts from three non-Jewish type 1 Gaucher disease patients but only in cell line B303 from a type 1 Ashkenazi Jewish Gaucher disease patient.

Processing of Acid β-Glucosidase in Fibroblasts from Patients with Type 2 Gaucher Disease

The heterogeneous patterns of acid β-glucosidase processing in fibroblasts from patients with type 2 (acute neuronopathic) Gaucher disease are shown in figures 6 and 7 for cell lines GM1260 and GM2627, respectively. Analysis of the processing of acid β-glucosidase from GM1260 fibroblasts revealed no processing of the 64-kDa form of the enzyme (fig. 6). In addition, the newly synthesized enzyme disappeared by 24 h. This same result was obtained in cell line GM877 (not shown). These results confirm those of Beutler and Kuhl (1986), using the same cell lines. In comparison, cell line GM2627 was able to process some of the 64 kDa enzyme to the 69-kDa form (fig. 7). In addition, some of the 64-kDa enzyme was still present after 24 h of chase. The 69-kDa form of the enzyme disappeared more rapidly than that in normal fibroblasts. No con-

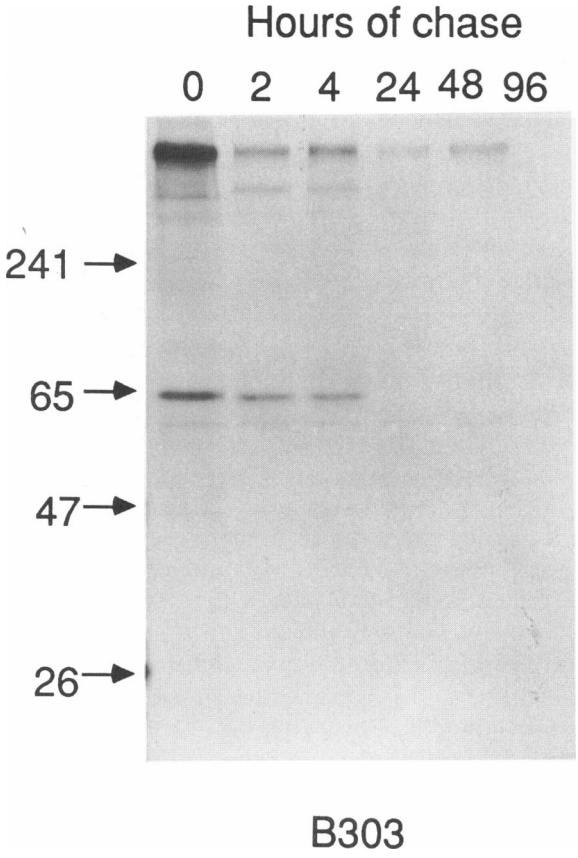

Figure 4 Time course of processing of acid β-glucosidase in atypical Ashkenazi Jewish type 1 Gaucher disease fibroblasts. Cell line B303 fibroblasts were labeled for 30 min with ^{35}S-methionine and were chased for the times indicated above each lane.

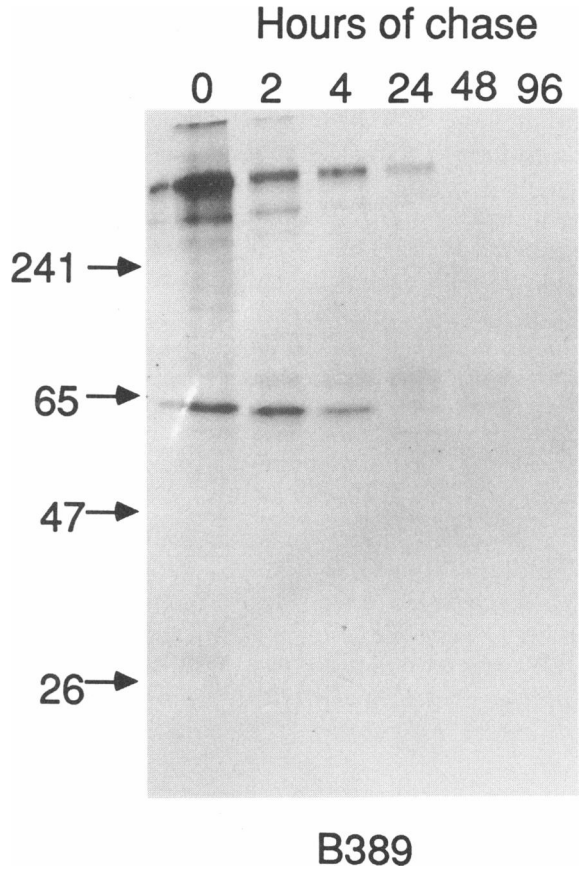

Figure 5 Time course of processing of acid β-glucosidase in fibroblasts from a non-Jewish type 1 Gaucher disease patient. Cell line B389 fibroblasts were labeled for 30 min with ^{35}S-methionine and were chased for the times indicated above each lane.

version to the normally present 59-kDa form was observed.

Discussion

Previous studies of human acid β-glucosidase have provided insight into the physical (Grabowski et al. 1985*b*, 1986), kinetic (Grabowski et al. 1985*a*, 1985*b*, 1986), and immunologic (Ginns et al. 1982; Pentchev et al. 1983; Beutler et al. 1984; Grabowski et al. 1985*a*) properties of this membrane-associated lysosomal hydrolase in normal and Gaucher disease tissues. However, until recently (Erickson et al. 1985; Beutler and Kuhl 1986; Tager et al. 1986) the posttranslational processing of this enzyme had been inferred from CRIM patterns on "western" blotting studies (Ginns et al. 1982; Fabbro et al. 1987). Such studies led to the speculation that the occurrence of allelic mutations resulted in differential and characteristic processing defects of the acid β-glucosidase in the subtypes of Gaucher disease (Ginns et al. 1982). Furthermore, the nature of the glycosidic modifications of the normal acid β-glucosidase remained unknown. Therefore, the present studies were designed to characterize the sequence of posttranslational modifications of acid β-glucosidase from normal fibroblasts and from several Gaucher disease variant fibroblasts. The fibroblasts from the specific patients in the present studies were selected, on the basis of the respective western blotting patterns (Fabbro et al. 1987), because they were thought to be either representative of a particular type 1 or type 2 allele(s) or representative of "exceptions" to those alleles.

On the basis of the results presented here, normal human acid β-glucosidase is synthesized initially as a

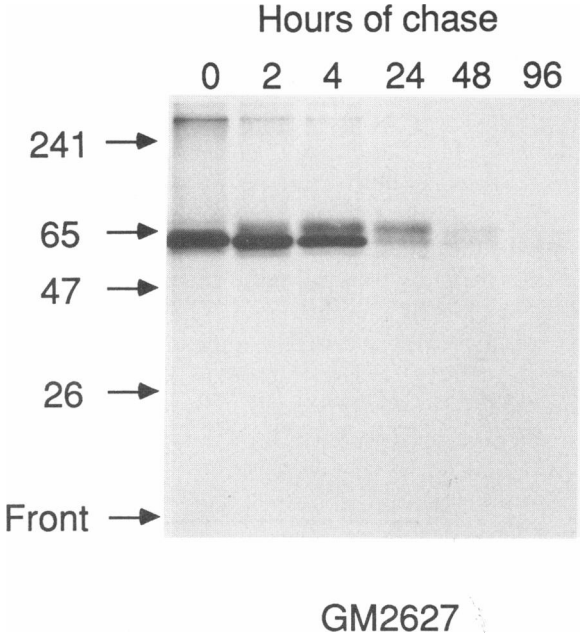

Figure 6 Time course of processing of acid β-glucosidase in typical type 2 Gaucher disease fibroblasts. Cell line GM1260 fibroblasts were labeled for 30 min with ^{35}S-methionine and were chased for the times indicated above each lane.

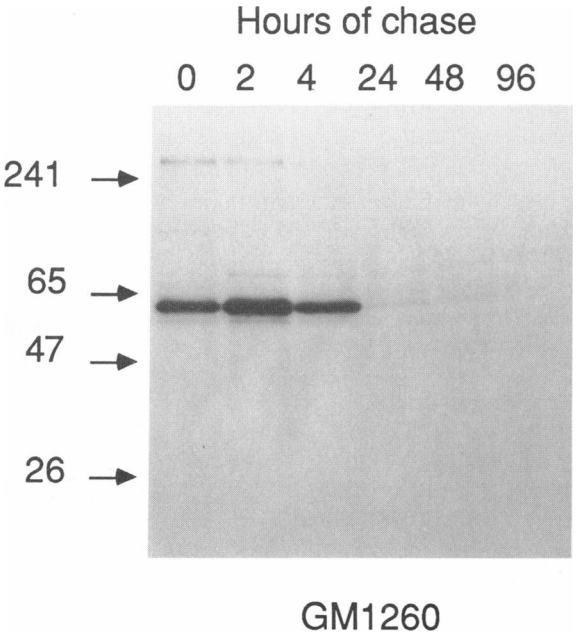

Figure 7 Time course of processing of acid β-glucosidase in atypical type 2 Gaucher disease fibroblasts. Cell line GM2627 fibroblasts were labeled for 30 min with ^{35}S-methionine and were chased for the times indicated above each lane.

glycosylated 64-kDa form. Removal of the oligosaccharides from the newly synthesized acid β-glucosidase by endo H digestion demonstrated that 55.3 kDa of this mass was due to the polypeptide chain. The additional 9 kDa of apparent molecular mass was due to the addition of four or five N-linked high-mannose-type oligosaccharides (Erickson et al. 1985). These results are in excellent agreement with the molecular weight (55.8 kDa) calculated from the amino acid sequence of the mature polypeptide (Sorge et al. 1985; Tsuji et al. 1986) and with a 2–3 kDa contribution from each high-mannose-type oligosaccharide to the apparent molecular weight. Remodeling of the oligosaccharides in the Golgi apparatus causes a further shift in the apparent molecular weight (from 64 kDa to 69 kDa). Neuraminidase digestion studies indicated that most of this shift was due to the addition of terminal sialic acid residues. Furthermore, the time course of this shift allowed us to estimate the $t_{1/2}$ (= 3 h) for the rate of transport of acid β-glucosidase from the rough endoplasmic reticulum to the Golgi apparatus.

An unusual feature of the posttranslational processing of the normal acid β-glucosidase was that its transport from the Golgi apparatus to the lysosomes was not associated with proteolytic processing as has been observed with several other lysosomal hydrolases (Hasilik 1980; Hasilik and Neufeld 1980; Steckel et al. 1983). Previous western blotting studies by Fabbro et al. (1987) demonstrated that three forms of acid β-glucosidase in normal fibroblasts contained N-linked oligosaccharides that were removed by N-Glycanase™ digestion. Removal of the oligosaccharides from all three forms resulted in one polypeptide with an apparent molecular weight of 55–56 kDa. The present studies extended these findings by demonstrating that removal of the high-mannose-type oligosaccharides from the newly synthesized (64-kDa) acid β-glucosidase, present in the rough endoplasmic reticulum, reduced its apparent molecular weight to 55.3 kDa. N-Glycanase™ treatment of the 69-kDa form present 24 h after synthesis also resulted in a 55.3-kDa polypeptide (fig. 2*B*). Thus, the newly synthesized form of β-glucosidase has the same molecular-weight polypeptide backbone as the form that has been fully processed in the Golgi apparatus and that has presumably been transported to the lysosomes. Both these polypeptides have the same apparent molecular weight as that previously seen in western blots of N-Glycanase™-treated acid β-glucosidase. Thus, in contrast to most other lysosomal enzymes, even the steady-state forms of acid β-glucosidase that reside in

the lysosome appear to escape proteolytic clipping (Hasilik 1980). This lack of proteolytic processing may be related to the close association of β-glucosidase with the lysosomal membrane.

In the fibroblasts from patients affected with the subtypes and variants of Gaucher disease, the processing of the mutant acid β-glucosidases was normal, absent, or inefficient (table 1). In fibroblasts from type 1 Ashkenazi Jewish Gaucher disease patients (except B303), the amount acid β-glucosidase was processed normally, confirming the results of Beutler and Kuhl (1986). In addition, the molecular forms of this mutant enzyme had nearly normal half lives, although the conversion to the 69-kDa forms was somewhat slower than normal. These results were consistent with previous studies with type 1 Ashkenazi Jewish Gaucher disease acid β-glucosidases, studies that detected about 30%–100% of normal amounts of immunologically cross-reactive material (Pentchev et al. 1983; Beutler et al. 1984; Grabowski et al. 1986) but that determined the enzymatic activity to be unstable (Grabowski et al. 1986). In comparison, the acid β-glucosidases in two type 2 cell lines (GM877 and GM1260) were normally stable for 4 h, but apparently were not processed beyond the 64-kDa glycosylated product. Previous deglycosylation studies provided direct evidence that these mutant enzymes had in fact lost their leader sequence and had received their high-mannose-type oligosaccharides in the rough endoplasmic reticulum (Fabbro et al. 1987). For any of these type 2 cell lines the mutant enzymes were sufficiently stable for processing to be detected, since by 2–4 h, over half of the newly synthesized normal enzyme had been processed in the Golgi apparatus. These results suggested that, compared with the normal enzyme, these mutant enzymes were either (1) far less efficiently transported to the Golgi apparatus, (2) not efficiently modified in the Golgi apparatus, or (3) extremely unstable after modification. In comparison, the defective acid β-glucosidase in the type 2 cell line, GM2627, was processed, albeit inefficiently, to the 69-kDa glycosylated form. The observation that GM2627 is not further processed to the 59-kDa form suggests that the mutant form of acid β-glucosidase fails to undergo post-Golgi oligosaccharide trimming. As the lysosome is the organelle in which such trimming would be most likely to occur, our data indicate that the acid β-glucosidase of GM2627 fibroblasts fails to reach the lysosomes or is unstable in the lysosomal environment. As the more typical type 2 fibroblasts (GM877 and GM1260) fail to accumulate even Golgi apparatus–modified forms of acid β-glucosidase (greater than 64 kDa), it may be that a common feature of type 2 Gaucher disease fibroblasts is a failure of acid β-glucosidase to reach the lysosomes and/or a marked instability of the enzyme in the lysosomes. In this regard, Tager et al. (1986) noted that incubation of type 2 Gaucher disease fibroblasts in medium containing leupeptin protected some acid β-glucosidase from degradation. Since this protease inhibitor is transported to the lysosomes, the protection from degradation afforded to mutant acid β-glucosidases by leupeptin suggests that a small percentage of the enzymes does reach the lysosomes but that it is degraded there.

Previous studies (Erickson et al. 1985; Beutler and Kuhl 1986; Fabbro et al. 1987) and the present investigations provide a general scheme for the posttranslational processing of normal acid β-glucosidase in cultured fibroblasts. However, the molecular masses of the acid β-glucosidase forms and that of the eventual final form of the normal enzyme are quite different in the various publications. However, when the same antibody preparation is used, the molecular masses of the acid β-glucosidase forms in the study by Fabbro et al. (1987) and in the present study were the same within 0.5%–2% for type 1 (Ashkenazi Jewish and non-Jewish [B389]) and type 2 (GM1260 and GM2627). This agreement indicates that our stringent immunoprecipitation meth-

Table 1

Summary of the Posttranslational Processing Steps Seen with β-Glucosidase from Normal and Gaucher Disease Fibroblasts

	Forms of β-Glucosidase			
Patient	I	II	III	
Normal	→		→	Normal
AW	→		→	Gaucher Type 1
B303	→			
B389	→			
GM1260	→			Gaucher Type 2
GM2627	→			

Note.—Immediately after synthesis, normal β-glucosidase contains solely high-mannose-type oligosaccharides which can be removed with endoglycosidase H. It has a molecular weight of 64 kDa (form I). Most of the oligosaccharides associated with β-glucosidase are converted to sialic acid terminal complex-type oligosaccharides in the Golgi apparatus, changing its apparent molecular weight to 69 kDa (form II). Over several days, the oligosaccharides are trimmed (presumably in the lysosomes), changing the apparent molecular weight of β-glucosidase to 59 kDa (form III).

odology did not cause an artifactual loss of any of the steady-state forms of these mutant forms of acid β-glucosidase.

The present results and those of previous western blotting studies (Fabbro et al. 1987) indicate that the allelic mutations in the acid β-glucosidase gene result in heterogeneous defects in the apparent processing of the resultant mutant enzymes in fibroblasts (table 1). It is important to note that the absence of apparent processing observed in most type 2, in the non-Jewish type 1, and in one of the Ashkenazi Jewish type 1 fibroblast cell lines used in this study indicated the inability to diagnostically discriminate between the neuronopathic and nonneuronopathic phenotypes by this method. These results indicate that the etiologies of the neuronopathic Gaucher disease phenotypes are not directly related to the abnormal processing of acid β-glucosidase seen in fibroblasts. Furthermore, the heterogeneity of the processing alterations found in the present studies and those of Beutler and Kuhl (1986) strongly suggests the existence of several different allelic mutations which result in the neuronopathic phenotype of Gaucher disease.

Indeed, several causal, missense mutations have been identified within and among the Gaucher disease variants (Tsuji et al. 1987, 1988; Graves et al. 1988; G. A. Grabowski, unpublished observations). Furthermore, relatively few Gaucher disease types 1, 2, and 3 patients were homozygous for the identified mutations within the β-glucosidase gene. It will be of considerable basic and clinical importance to correlate the processing defects observed here with the genetic lesions. Such correlations will require analyses of the fate of β-glucosidase expressed from mutagenized cDNAs for individual alleles in the appropriate cell systems.

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