Novel mutations (Asn 484 Lys, Thr 500 Ala, Gly 438 Glu) in Morquio B disease

Richard D. Bagshawa, Sunqu Zhangb, Alina Hineka, Marie-Anne Skomorowskia, Donald Whelanb, Joe T.R. Clarkeb, John W. Callahanb,d,*

aDepartment of Pediatric Laboratory Medicine and Pathobiology, The Hospital for Sick Children, University of Toronto, Toronto, Canada
bDepartment of Pediatric Laboratory Medicine and Biochemistry, The Hospital for Sick Children, University of Toronto, Toronto, Canada
cDepartment of Genetics, McMaster University, Hamilton, ON, Canada
dDepartment of Pediatric Laboratory Medicine and Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Canada

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Abstract

Primary deficiency of β-galactosidase results in GM1 gangliosidosis and Morquio B disease. Of the more than 40 disease-causing mutations described in the Gal gene to date, about 75% are of the missense type and are scattered along the length of the gene. No single, major common mutation has been associated with GM1 gangliosidosis. However, a Trp 273 Leu mutation has been commonly found in the majority of patients with Morquio B disease defined genotypically to date.

We now report three new mutations in three Morquio B patients where the Trp 273 Leu mutation is absent. Two of the mutations, C1502G (Asn 484 Lys) and A1548G (Thr 500 Ala), were found in twins (one male, one female) who display a mild form of Morquio B disease and keratan sulfate in the urine. In their fibroblasts, residual activity was 1.9% and 2.1% of controls. On Western blots, the 84-kDa precursor and the 64-kDa mature protein were barely detectable. The occurrence of a 45-kDa degradation product indicates that the mutated protein reached the lysosome but was abnormally processed. In the third case, we identified only a G1363A (Gly 438 Glu) mutation (a major deletion on the second allele has not been ruled out). This female patient too displays a very mild form of the disease with a residual activity of 5.7% of control values. In fibroblasts from this case, the 84-kDa precursor and the 45-kDa degradation product were present, while the mature 64-kDa form was barely detectable. The occurrence of these three mutations in the same area of the protein may define a domain involved in keratan sulfate degradation.

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1. Introduction

Morquio disease (MPS IV) occurs in two types (A and B) due to deficiencies in two separate genes. The disorder is characterized as a massive dysostosis multiplex with storage of keratan sulfate but without proven primary central nervous system involvement demonstrable either neuropathologically or biochemically. Patients with type A are generally more severely involved clinically, whereas the B form tends to be milder with a later onset of clinical symptoms [1,2]. Morquio A disease, the more common form of the two, arises from mutations in the gene encoding a 6-sulfate sulfatase that is specific for residues found in keratan sulfate (both galactose 6-sulfate and N-acetylgalactosamine 6-sulfate) and in chondroitin 6-sulfate (as N-acetylgalactosamine 6-sulfate). β-Galactosidase activity is normal in type A patients. Morquio disease, type B (MPS IVB) arises from deficiency in β-galactosidase where the activity of the 6-sulfate sulfatase is normal. A deficiency in the same β-galactosidase arising from the same Gal gene is also the primary defect in GM1 gangliosidosis. The latter is clinically heterogeneous and is characterized as a neuronal lipid storage disease, with GM1 ganglioside as the primary storage product in central nervous system neurons (see Ref. [1] for a recent review of this topic).
The β-galactosidase gene, located on chromosome 3p21.33 [3–5] is 62.5 kb in length and composed of 16 exons [6,7]. Two related proteins arise by alternative splicing of the primary β-galactosidase transcript. The most abundant product is lysosomal β-galactosidase that we have shown is catalytically active in its precursor form. The second protein, originally called the β-galactosidase-like protein, does not display β-galactosidase activity and has been characterized by us as the elastin binding protein (EBP) of the elastin binding receptor. It arises by alternative splicing whereby the sequence encoded by exons 3, 4 and 6 is missing, a frameshift occurs in exon 5 (which encodes a unique 32-amino-acid sequence) [6] and the reading frame is restored at the start of exon 7. Except for this frameshifted unique sequence, the primary structure of the EBP is identical to β-galactosidase. However, the latter is localized to the cell surface but not to lysosomes [6,8].

Mutations are spread throughout the gene, 70% of which occur simultaneously in both β-galactosidase and the EBP. Most are of the missense type and give rise to GM1 gangliosidosis. Most patients with GM1 gangliosidosis are heteroallelic with different mutations on each parental chromosome and missense mutations affecting some codons (such as those affecting Arg 59, Arg 201, Arg 208, Pro 263, Arg 482) have been noted more frequently than others. In contrast, a major common mutation, Trp 273 Leu, has been found in the majority of the 17 Morquio B patients that have been defined [9,10] to date. It is noteworthy that no patient with one copy of the Trp 273 Leu mutation has presented with GM1 gangliosidosis; all such patients have the clinical and biochemical phenotype of Morquio B disease.

β-Galactosidase cleaves substrates by formation of a galactosylated-amino acid intermediate (the catalytic nucleophile), with subsequent release of the galactose and retention of β-configuration. This mechanism predicts that at least two acidic residues are involved in the catalytic mechanism. We identified the galactosylated residue as Glu 268 [11] and our recent work has identified Asp 332 as a candidate for the second acidic residue in the catalytic mechanism [12]. Because Trp 273 Leu has been the only mutation strictly associated with Morquio B disease to date, it has been postulated to play an essential role in the hydrolysis of keratan sulfate. However, to date, there have been no studies directly examining this question. It has also been proposed that the Trp 273 Leu mutation affects the ability of β-galactosidase to join a lysosomal complex also containing cathepsin A, neuraminidase and GalNAc-6-sulfate sulfatase, the formation and function of which is poorly understood. Thus, the role of Trp 273 in complex binding and hydrolysis of natural substrates, particularly the keratan sulfates, is ill defined.

In the present work, we describe new mutations, Asn 484 Lys and Thr 500 Ala in a set of fraternal twins and Gly 438 Glu in a third unrelated patient, all of whom display the clinical and biochemical features of Morquio B disease. The Trp 273 Leu mutation is absent from these patients. The present data have appeared in abstract form [13].

2. Materials and methods

2.1. Clinical findings

The patients included in this study satisfy the criteria for Morquio disease; namely, systemic bone disease without primary involvement of the central nervous system (defined as retarded developmental milestones, retarded intellectual development, presence of spasticity and seizures). Family 1. The first of the twins presented at age 9 years with an abnormal gait and increased fatigability. On examination, he was not dysmorphic, there was no hepatosplenomegaly, and the neurological exam was normal. Some hip weakness was noted. X-rays of the spine revealed platyspondyly with vertebral beaking typical of MPS IV. Keratan sulfate was isolated from his urine at this time. Residual leukocyte β-galactosidase activity was <2.5% of the normal level (in all aspects of this study activity was assayed with the fluorimetric 4-methylumbelliferyl-β-D-galactopyranoside substrate as previously described [14]). By age 11 years, odontoid dysplasia, thoracolumbar scoliosis, and genu valgum typical of the disease were evident. M.S., his twin, was essentially normal at age 9.5 years. Keratan sulfate was isolated from her urine also and again residual leukocyte β-galactosidase activity was <2.5% of the normal level. A mild dorsal scoliosis was noted at age 11 years. At age 18 years, her neurological status was within normal limits, she had an unsteady gait but remained ambulatory, and was otherwise healthy. Their early developmental history was uninformative and the parents (Portuguese) are unrelated. Both patients now in their late-20s are educated both having successfully completed post-secondary education, their neurological status remains within normal limits, but their skeletal abnormalities seriously impair their ambulatory capabilities.

Family 2. This female was diagnosed at the age of 8 years by the presence of keratan sulfate in the urine and a deficiency of leukocyte β-galactosidase (<1% of the normal value) was noted. At 18.5 years, she was short (147.5 cm), had a mild dorsal scoliosis, denied pain in the joints or back, and she had mild limitation of movement of neck, spine, hips and shoulders. Her neurological exam was unremarkable, she was judged to be somewhat dull, and on psychological assessment, her IQ was judged to be borderline normal. Corneal clouding was noted at this time, and the cardiovascular system was normal. This patient married and is now in her early 30s but she and her parents now have been lost to follow-up.

2.2. Fibroblast culture

Fibroblast strains were established for diagnostic studies that were subject to informed consent. Cells from a case of
Morquio disease, type B (GM01602; mutations TG817/818CT; Trp 273 Leu and G1445A; Arg 482 His), and GM1 gangliosidosis (GM05652A; homozygous for mutation C1101T; Arg 351 ter) were from the NIGMS Human Genetic Mutant Cell Repository. In general, skin fibroblasts were grown in α-MEM with penicillin (100 U/ml) and streptomycin (100 μg/ml), supplemented with 10% fetal calf serum (FCS) at 37 °C in 5% CO₂ atmosphere until confluent. All cell cultures used in this work were negative for the presence of mycoplasma contamination, as analysed by a PCR-based method. In the leupeptin experiments, cells were grown in α-MEM medium (in the presence or absence of 20 μM leupeptin) with 10% FBS at 37 °C in 5% CO₂.

2.3. Isolation of genomic DNA

Genomic DNA was isolated from harvested cultured skin fibroblasts suspended in lysis buffer (100 mM NaCl, 10 mM Tris–Cl pH 8, 25 mM EDTA pH 8, 0.5% SDS, and 0.1 mg/ml proteinase K) by freeze/thawing (10 ×) the cells in a dry ice/ethanol bath. An equal volume of phenol/chloroform was then added, and DNA was precipitated by adding 1/2 volume of 7.5 M ammonium acetate and 2 volumes of 100% ethanol. The DNA pellet was rinsed with 70% ethanol and dissolved in 10 mM Tris pH 8, 0.1 mM EDTA pH 8 (TE buffer).

2.4. PCR and restriction enzyme analysis

Exons 2–16 were amplified from 500 ng of patient or normal genomic DNA. The primers used to amplify the appropriate exons of the β-galactosidase gene and those used for site-directed mutagenesis are based on the published intron/exon junction sequences [7]. DNA amplification reactions were performed in 100 μl total volume containing: 100 ng of each primer, 10 units of Taq polymerase (Promega), 0.5 mM each of dCTP, dGTP, dTTP, and dATP, in 10 mM Tris–Cl pH 8, 0.1 mM EDTA pH 8 (TE buffer). The reaction conditions were 1 min at 94 °C (denaturation), 1 min 10 s at 56 °C (annealing), and 1 min at 72 °C (extension) for 30 cycles using the RoboCycler 40 (Stratagene) thermal cycler. PCR products were run on a 1% agarose gel to check for insertions or deletions before SSCP analysis was performed.

2.5. SSCP analysis

SSCP analysis was performed at room temperature and at 4 °C with 6% polyacrylamide gels in 0.5 × TBE running buffer containing 5% glycerol (40 W) as described by Refs. [17,18]. The gels fixed in 50% methanol and 10% acetic acid solution for 1 h were washed three times in distilled water for 15 min and then stained in a solution containing equal volumes of 0.5 M sodium carbonate and 10 mM silver nitrate–25 mM ammonium nitrate–3.5 mM tungstosilicic acid–0.8% formalin for 10–30 min. The reaction was stopped by the addition of 10% acetic acid.

2.6. DNA sequencing

Direct sequencing of PCR products in both directions followed the Amersham 32P-thermossequenase cycle sequencing protocol.

2.7. Western blot analysis

Cells were harvested by scraping in phosphate-buffered saline and lysed by sonication as described earlier. Thirty micrograms of fibroblast protein was analysed using SDS-PAGE. Samples were reduced by boiling for 5 min in 62.5 mM Tris, 3% SDS, 10 mM DTT, and were loaded onto an 8% Bio-Rad Mini Gel SDS-polyacrylamide system as described earlier [12]. Proteins were transferred overnight to nitrocellulose, and the Western blot was probed using a 1:1500 dilution of the polyclonal rabbit IgG (P-Gal) raised against purified human β-galactosidase precursor. This multipieptic antibody reacts with all known forms of the wild-type and mutant protein including their degradation products [12,19]. Immunoblots were developed using the enhanced chemiluminescence system (ECL-Western Blot, Amersham).
2.8. Immunolocalization of β-galactosidase in intracellular compartments

Histochemical staining was by incubation of fixed permeabilized cells with indolyl-β-galactoside [20]. Cells were grown in α-MEM medium (in the presence or absence of 20 μM leupeptin) with 10% of FBS at 37 °C in 5% CO₂ on glass cover slips, and after 48 h, the cells were fixed and gently permeabilized with 100% cold methanol at −20 °C for 30 min. The fixed cells were then washed in phosphate-buffered saline, blocked with 1% bovine serum albumin, and incubated for 1 h with rabbit IgG raised against purified human β-galactosidase precursor as above (diluted 1:200). The secondary antibody, a green fluorescein-labeled goat-anti-rabbit IgG F(ab')₂, diluted 1:100, was then added for 1 h, either alone or in combination with a 1:10,000 dilution of propidium iodide, which in addition to nuclear DNA also stains cytoplasmic RNA, and RNA bound to the rough ER thereby marking the position of the ER with the red fluorescence. The cells were then washed three times with phosphate-buffered saline and mounted with elvanol [21]. In control cultures, the preimmune rabbit IgG was substituted for the primary antibody.

3. Results

3.1. Identification of the mutations

Direct sequencing of PCR products was used to identify mutations in exons 2–16 using intron/exon primers as described by Morreau et al. [7]. Both patients in Family 1 were found to be heterozygous for two mutations, a C1502 → G transversion in exon 14 which converts Asn 484 to Lys (Fig. 1a), and an A1548 → G substitution in exon 15 which converts Thr 500 to Ala (Fig. 1b). The patient in Family 2 was homozygous for a G1363 → A substitution which converts Gly 438 to Glu in exon 13 (Fig. 1c). We did not detect the nucleotide changes noted in the Morquio patients defined in this study using genomic DNA samples from 30 normal fibroblast lines supporting the assignment of these mutations as disease-causing and not naturally occurring polymorphisms. The Trp 273 Leu mutation commonly found in patients with Morquio B disease was confirmed in the cell repository Morquio B line used as the positive control as was the Arg 482 His mutation. The Trp 273 Leu mutation was not present in any of the current patients.

Fig. 1. Identification of mutations in Families 1 and 2. Genomic DNA was isolated as described. One mutation was detected in each of exon 14 and 15 in Family 1 and in exon 13 in Family 2. Genomic fragments were amplified from 500 ng of patient or normal DNA using primers based on intronic/exonic sequences and using the amplification conditions as described in the Materials and Methods. Direct sequencing of PCR products in both directions followed the Amersham 33P-thermosquenase cycle sequencing protocol. In Family 1, we noted in exon 14 a C1502G transversion which changed Asn 484 to Lys (Panel a), and an A1548G substitution converting Thr 500 to Ala (Panel b) in exon 15. In Family 2, the only mutation noted was a G1363A substitution converting Gly 438 to Glu (Panel c) in exon 13.
3.2. Level of β-galactosidase protein

The total β-galactosidase activity in fibroblast extracts from our Morquio B patients was 2.1% and 1.88%, respectively, for the twins in Family 1, and 5.67% in the patient from Family 2 (Table 1). This compares favourably to the values obtained for the positive Morquio B control from the cell repository (1.2%). All of these residual activities are higher than the extremely low level of activity in the GM1 gangliosidosis cells (< 0.5% of the control) included in the study. To assess the impact of these mutations on the level of β-galactosidase protein in these cells, we used our well-characterized rabbit polyclonal antibody in Western blot analyses. We detected very small but comparable to normal amounts of the 84-kDa precursor in cells from the Family 1 patients (Fig. 2, lanes 1 and 6), and the case from the cell repository (Fig. 2, lane 3), while in the third patient (from Family 2), the precursor was more abundant (Fig. 2, lane 2). The processed, mature 64-kDa polypeptide, the most abundant form of the enzyme, was easily detected as expected in normal cells. The mature protein was decreased in the Family 1 fibroblasts (Fig. 2, lanes 1 and 6) to the levels seen in the GM1 gangliosidosis cells (Fig. 2, lane 5), but was found at a higher level in fibroblasts from Family 2 (Fig. 2, lane 2). On the other hand, fibroblasts from the positive Morquio B control displayed significant amounts of the 64-kDa mature protein (Fig. 2, lane 3). It is noteworthy that in all fibroblasts except those from the patient with the stop codon mutation (Fig. 2, lane 5), prominent degradation products in the range of 45 kDa were detectable. However, the relative abundance of these forms was not constant. For example, as in the normal cell line, Family 1 and 2 cells appeared to have a single major degradation product compared to at least two for the cell repository case and three major bands in one of the GM1 gangliosidosis cases. The relative abundance of all forms of the protein in the Morquio B cases was substantially higher than that found in the case of GM1 gangliosidosis used as a control but was still lower than that noted in the control cell line. Maturation of the precursor to the mature protein and formation of these degradation products arise exclusively from lysosomal digestion since as we have previously shown in 1-cell fibroblasts, where the precursors are not localized into lysosomes due to the absence of the mannose 6-phosphate targeting signal, that the enzyme is not

![Fig. 2. Western blot of fibroblast lysates from Morquio B and GM1-gangliosidosis patients. Thirty micrograms of total cellular protein was analysed by SDS-PAGE and probed using our well-characterized antibody that cross-reacts with the 84-kDa precursor and the 64-kDa mature forms of β-galactosidase. Lane 1 is Morquio B, sibling 1 of Family 1; Lane 2, is the case from Family 2; Lane 3 is a Morquio B cell line (GM01602) from the NIMCGS repository; Lane 4 is a normal control; Lane 5 is a case of GM1 gangliosidosis, homozygous for an Arg351STOP mutation; Lane 6 is sibling 2 of Family 1; Lane 7 corresponds to wild-type β-galactosidase expressed by CHO cells, as described earlier [14] and demonstrates the location of the 84-kDa precursor and the 64-kDa processed mature form of the enzyme.](image)

![Fig. 3. Western blot of lysates from Morquio B patients incubated in the presence of leupeptin. Thirty micrograms of total cellular protein was analysed by SDS-PAGE and probed using our well-characterized antibody that cross-reacts with the 84-kDa precursor, the 64-kDa mature form and the major degradation product of β-galactosidase at 45 kDa. Lane 1 is Morquio B, sibling 2 of Family 1; Lanes 2 and 3 is Morquio B, Family 2; Lanes 4 and 5 is Morquio B cell line (GM01602) from the NIMCGS repository; Lanes 6 and 7 is a normal control. Lanes 1, 3, 5, 5 and 7 are lysates from cells grown in the presence of leupeptin for 48 h; lanes 2, 4, 6 are from cells grown in the absence of leupeptin.](image)

Table 1

<table>
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<th>Cell line mutations</th>
<th>Specific activitya</th>
<th>Western blot result</th>
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<tr>
<td><strong>Family 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sibling 1 Asn 484 Lys Thr 500 Ala</td>
<td>30 18,220</td>
<td>some precursor, little mature β-gal, degradation products</td>
</tr>
<tr>
<td>sibling 2 Asn 484 Lys Thr 500 Ala</td>
<td>27 13,602</td>
<td>some precursor, little mature β-gal, degradation products</td>
</tr>
<tr>
<td><strong>Family 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1 Gly 4328 Glu homozygous</td>
<td>81 22,248</td>
<td>abundant precursor, little mature β-gal, degradation products</td>
</tr>
<tr>
<td><strong>Cell repository</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tpr 273 Leu Arg 482 His</td>
<td>17 11,772</td>
<td>some precursor, some mature β-gal, degradation products</td>
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<tr>
<td><strong>GM1 gangliosidosis</strong></td>
<td>Arg351STOP</td>
<td>3 21,690</td>
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<tr>
<td><strong>Normal</strong></td>
<td>none known</td>
<td>1429 17,419</td>
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*a Specific activity is nanomoles per hour per milligram of protein.*
processed but rather degraded [19]. These findings are summarized in Table 1. Because it could be argued that the mutant proteins in the Morquio B lines reached the lysosomes in normal abundance but were quickly degraded, we examined the level of β-galactosidase proteins in cells incubated in the presence of leupeptin (Fig. 3). We previously showed in galactosialidosis fibroblasts, where the β-galactosidase deficiency is a secondary manifestation of the primary deficiency in cathepsin A/protective protein, incubating the fibroblasts in the presence of leupeptin restored β-galactosidase to normal levels but did not affect processing to the mature enzyme [19]. In this instance, exposure of Morquio B cells to leupeptin did not effect a rise in β-galactosidase activity regardless of the nature of the mutations (Table 2). No substantial change in the level of β-galactosidase proteins (precursor, mature or degraded forms) were noted in the Morquio B lines with the Asn 484 Lys/Thr 500 Ala or the Gly 438 Glu mutations in the presence of leupeptin. In contrast, in the control lines used (in both the Trp 273 Leu/Arg 482 His Morquio B line and in the normal), leupeptin treatment resulted in a net increase of β-galactosidase proteins. Since leupeptin did not result in a rise in the enzyme protein in the presence of the Asn 484 Lys/Thr 500 Ala or the Gly 438 Glu mutations, it implies that the mutated protein does not reach the lysosome but rather is degraded in a pre-lysosomal compartment. In support of this, we undertook immunofluorescence localization studies. Here, β-galactosidase protein was found primarily in the perinuclear area (i.e. the endoplasmic reticulum) with some immunoreactivity dispersed in organelles distant from the nucleus (data not shown).

<table>
<thead>
<tr>
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<th>Specific activity (nmol/h/mg of protein)</th>
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<tr>
<td></td>
<td>No leupeptin</td>
</tr>
<tr>
<td>Control</td>
<td>681.3</td>
</tr>
<tr>
<td>Morquio B, Family 1, Case 1</td>
<td>8.9</td>
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<tr>
<td>Morquio B, Family 2</td>
<td>19.9</td>
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<tr>
<td>Morquio B, cell repository</td>
<td>7.8</td>
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<tr>
<td>GM1 gangliosidosis</td>
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Cultured skin fibroblasts were incubated for 48 h in the presence of 10 mM leupeptin, after which the cultures were harvested, and processed as described.

4. Discussion

Novel missense mutations (Thr 500 Ala, Asn 484 Lys, and Gly 438 Glu) have been found in three patients in two families who display a mild form of Morquio B disease (primary β-galactosidase deficiency with keratan sulfate storage). Subsequent to our conference report [13], Paschke et al. [10] found the same A1532C, Thr 500 Ala mutation in another mild patient diagnosed at the age of 24 years, but they did not examine the impact on the level of cross-reacting material. In the presence of the Thr 500 Ala and Asn 484 Lys mutations, minimal amounts of precursor (84 kDa) and mature (64 kDa) forms of β-galactosidase were detected, the most abundant form of β-galactosidase protein being a 45-kDa degradation product. With the Gly 438 Glu mutation, both the precursor and the 45-kDa degradation product were noted but again only minimal amounts of the mature form of the enzyme were detected. Incubation of these cells with leupeptin, a potent thiol protease inhibitor, did not affect the level of residual β-galactosidase protein nor did it have any effect on the level of residual activity. On the other hand, with leupeptin treatment, there was a noticeable increase in the level of β-galactosidase precursor protein in control cells and in cells from the Morquio B patient heterozygous for Trp 273 Leu and Arg 482 His mutations, but no change in enzyme activity was observed in the latter. Consistent with this are our initial unpublished findings that show the mutant protein containing the Trp 273 Leu mutation to be catalytically defective at least with synthetic substrates. The failure to alter the level of β-galactosidase protein in the presence of leupeptin suggests that the proteins bearing the Thr 500 Ala, Asn 484 Lys, and Gly 438 Glu mutations do not reach the lysosome and are likely degraded in the endoplasmic reticulum, as we have previously shown for missense mutations causing GM1 gangliosidosis [12].

In normal cells, upon arrival in the endosomal–lysosomal compartment, β-galactosidase associates with Neuraminidase, protective protein (cathepsin A), and N-acetylglucosamine 6-sulfate sulfatase [22] to form a stable complex responsible for the hydrolysis of GM1 ganglioside and keratan sulfate. Complex formation is absolutely essential for β-galactosidase stabilization and for its normal posttranslational processing from the precursor to its mature form. Pshezhetsky and Potier [22] demonstrated that the complex also contained N-acetylgalactosamine 6-sulfate sulfatase, the enzyme deficient in Morquio disease, type A, and that its activity was reduced in galactosialidosis. This was correlated with elevated levels of keratan sulfate in the urine of galactosialidosis patients. These data indicate that digestion of keratan sulfate is intimately associated with and dependent upon both complex formation and correct processing of the enzyme. In earlier studies using I-cell fibroblasts, we showed that β-galactosidase precursor is degraded to a low molecular weight fragment (about 18 kDa), but no larger intermediate forms were detected. This non-lysosomal proteolysis of β-galactosidase arises due to its mislocalization reflecting the enzyme deficiency in I-cell disease. Because the mutations described here resulted in formation of significant amounts of the 45-kDa degradation products, it is clear that a substantial amount of the mutant enzyme reached the lysosome. However, little productive processing of the precursor to the mature 67-kDa form was noted suggesting that these mutations likely affect the protein’s ability to associate correctly with the lysosomal complex, a crucial step for the enzyme’s maturation and for keratan sulfate digestion. On the other hand, in the cell line with the Trp 273 Leu mutation,
measurable productive processing of the precursor to the mature protein occurred. These differences suggest that keratan sulfate catalysis is impaired in the presence of the Thr 500 Ala, Asn 484 Lys and Gly 438 Glu mutations because formation of the complex is abnormal, whereas with the Trp 273 Leu mutation, complex formation occurs, but the enzyme may be catalytically defective with respect to keratan sulfate degradation. Therefore, the occurrence of these new mutations in an area bounded by Gly 438–Thr 500 may define a region of β-galactosidase that is pivotal in its binding to the complex either alone or in conjunction with keratan sulfate binding and further work is required to examine this.

We have documented that the alternatively spliced form of β-galactosidase is identical to the 67-kDa EBP, the major function of which is to associate with a cell surface elastin binding receptor and to assist in the delivery of tropoelastin to form the extracellular matrix[8,23,24]. The elastin binding receptor is composed of EBP, and two other proteins, which we have shown to be neuraminidase, and protective protein (cathepsin A). Release of newly secreted tropoelastin molecules from the association with EBP occurs upon interaction between it and galactosugar moieties protruding from the carbohydrate chains of glycoproteins thus facilitating formation of the microfibrillar scaffold of elastic fibers. In parallel studies on fibroblasts from the Morquio B patients used in the present work and from patients with GM1 gangliosidosis, we have found that cell lines from patients with Morquio disease type B have decreased capacity to assemble elastic fibers, whereas most GM1 gangliosidosis do. With Morquio disease type B have decreased capacity to assemble elastic fibers, whereas most GM1 gangliosidosis do.

References


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