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Identification of a variant of mucolipidosis III (pseudo Hurler polydystrophy): A catalytically active *N*-acetylglucosaminyl-phosphotransferase that fails to phosphorylate lysosomal enzymes

(I-cell disease/glycoproteins/mannose 6-phosphate)

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ABSTRACT **Fibroblasts from patients with I-cell disease (mucolipidosis II) or with pseudo Hurler polydystrophy (mucolipidosis III) are markedly deficient in UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase. As a consequence, the common phosphomannosyl recognition marker of acid hydrolases is not generated, and these enzymes are not targeted to lysosomes. We have developed a sensitive assay for the transferase that uses α-methyl mannoside as an acceptor, and this has allowed us to distinguish between fibroblasts from these two types of patients. The enzyme activity is less in the former than in the latter (<0.4–2.0 pmol/mg per hr vs 2.9–39.4). This may provide an explanation for the difference in clinical severity between the two syndromes. However, in two siblings with pseudo Hurler polydystrophy (GM 3391 and GM 3392), the enzyme activity was normal when assayed by using α-methyl mannoside as acceptor whereas it was low when assayed with endogenous glycoprotein acceptors or with human placental β-hexosaminidase A. The apparent K_m values of the mutant enzyme toward α-methyl mannoside, high-mannose oligosaccharides, and UDP-GlcNAc were not different from those of the normal enzyme. Mixing experiments demonstrated that the mutant fibroblasts contained endogenous acceptors and were free of inhibitors. We conclude that the *N*-acetylglucosaminylphosphotransferase in the mutant fibroblasts has normal catalytic activity but is defective in the ability to recognize lysosomal enzymes as specific substrates for phosphorylation. This variant form of pseudo Hurler polydystrophy demonstrates the biological importance of this recognition mechanism in the generation of the phosphomannosyl marker.**

Acid hydrolases are a heterogeneous group of proteins that acquire a common phosphomannosyl recognition marker which is responsible for targeting them to their common destination, the lysosomes (1–11). This posttranslational modification of the high-mannose-type oligosaccharides of lysosomal enzymes is generated by the sequential action of two enzymes: UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase [referred to herein as *N*-acetylglucosaminylphosphotransferase (GlcNAc*P*Tase)] and α-*N*-acetylglucosaminyl phosphodiesterase. The first enzyme catalyzes the transfer of GlcNAc 1-phosphate to the 6 position of mannose residues on high-mannose-type oligosaccharides; the second enzyme removes the outer GlcNAc residues to generate a phosphomonoester (12–18). We have recently shown that fibroblasts from patients with I-cell disease (mucolipidosis II) or pseudo Hurler polydystrophy (mucolipidosis III) are markedly deficient in the first enzyme of this sequence (19). This explains why both diseases are characterized by decreased intracellular activities of many lysosomal enzymes and markedly increased levels of the same enzymes in the body fluids. Similar findings have been reported by Hasilik *et al.* (20).

In this report, we describe a variant form of pseudo Hurler polydystrophy in which the GlcNAc*P*Tase has normal catalytic activity toward an artificial substrate but is markedly deficient in the phosphorylation of lysosomal enzymes. This variant enzyme appears to be defective in the identification of lysosomal enzymes as specific substrates for phosphorylation.

METHODS

Materials. Materials were obtained from the following sources: 3a70 scintillation mixture and Triton X-100 (Research Products International, Elk Grove Village, IL); concanavalin A (Con A)-Sepharose (10 mg/ml) (Pharmacia); [γ-^{32}P]ATP (2000 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels) and [2-^{3}H]mannose (10 Ci/mmol) (New England Nuclear); α-minimum essential medium (Flow Laboratories, Rockville, MD); fetal bovine serum, penicillin, and streptomycin (GIBCO); QAE-Sephadex (Q-25-120), α-methyl D-mannoside, UDP-GlcNAc, and other reagents (Sigma). The following materials were prepared as previously described: [β-^{32}P]UDP-GlcNAc (15), $Man_9GlcNAc$, $Man_8GlcNAc$, $Man_7GlcNAc$, $Man_6GlcNAc$, $Man_5GlcNAc$, and ManGlcNAc standards (21) and purified human IgM (22). [^{32}P]Mannose 6-phosphate was prepared enzymatically from mannose and [γ-^{32}P]ATP by using yeast hexokinase. The product was isolated by paper chromatography on Whatman 3MM.

High-mannose-type oligosaccharides were prepared from IgM glycopeptides as follows. Glycopeptide peak II was isolated from IgM Wa exactly as described (22) and chromatographed on a column of Bio-Gel P-6 with 0.1 M NH_4HCO_3 as the eluting buffer. The column fractions were monitored by using the phenol/sulfuric acid method (23) for detection of hexoses. The glycopeptide peak was pooled, concentrated, desalted, and treated with endo-β-*N*-acetylglucosaminidase H (endo H) for 72 hr to release the high-mannose-type oligosaccharides. The digest was rechromatographed over the same Bio-Gel P-6 column, and the released oligosaccharides, which now migrated in a more retarded position, were pooled and desalted.

Buffers. The following buffers were used. Buffer A: 20 mM Tris·HCl, pH 7.45/155 mM NaCl. Buffer B: 50 mM Tris·HCl, pH 7.5/0.25 mM dithiothreitol/1 mM $CaCl_2$/1 mM $MgCl_2$/0.75% Triton X-100. Buffer C: buffer A with 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.02% sodium azide.

Enzymes. *Streptomyces plicatus* endo H was from Miles. Jack bean α-mannosidase was prepared as described by Li and

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Abbreviations: GlcNAc*P*Tase, *N*-acetylglucosaminylphosphotransferase; endo H, endo-β-*N*-acetylglucosaminidase H; Con A, concanavalin A.

Li (24). Purified human placental β-hexosaminidase A was generously provided by Arnold Miller (University of California at San Diego). Rat liver GlcNAc*P*Tase was purified 110-fold by using subcellular fractionation, differential detergent extraction, and DEAE-cellulose chromatography (to be described elsewhere).

Analytical Methods. Protein was determined by the method of Lowry; 0.5% $NaDodSO_4$ was used to eliminate interference caused by Triton X-100 (25). Descending paper chromatography for the separation of sugars and oligosaccharides was carried out in ethyl acetate/pyridine/acetic acid/water, 5:5:1:3. The treatment of oligosaccharides with jack bean α-mannosidase and of glycopeptides with endo H has been described (14). Desalting of samples was carried out on columns of Sephadex G-25 (1.0 × 30 cm). $NaDodSO_4$/polyacrylamide gel electrophoresis was performed as described (26), except that the concentration of acrylamide was 10%. Autoradiography was performed at −70°C for 36 hr with Kodak X-Omat AR film and a Cronex Lightning-Plus (Dupont) intensifying screen.

Cells. Fibroblasts from patients with I-cell disease or pseudo Hurler polydystrophy were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ), except for cells from T.M., which were provided by William Sly (Washington University). Eugene Bauer and Nancy Baenziger (Washington University) kindly provided the normal fibroblasts. The conditions for the growth, maintenance, and passage of cells were exactly as described (19).

Radiolabeling of Fibroblasts. Confluent cultures of fibroblasts in 150-mm Petri dishes were washed with 10 ml of glucose- and serum-free medium and then labeled with 1 mCi of [^{3}H]mannose for 2 hr in 10 ml of the same medium. The radioactive medium was then removed, and the label was chased for 30 min with 20 ml of complete serum-free medium containing 20 mM mannose. The cells were then chilled with ice-cold buffer A, harvested by scraping with a rubber policeman, and washed with 10 ml of the same buffer. The cell pellet was sonicated and digested with Pronase as described (12). The soluble glycopeptide material was diluted with 6 vol of buffer C, for application to Con A-Sepharose.

Characterization of the High-Mannose-Type Oligosaccharides. A portion (5%) of the labeled glycopeptide material was applied to a 1-ml column of Con-A Sepharose. The column was washed with 15 ml of buffer C and eluted with 10 ml of buffer C containing 10 mM α-methyl glucoside, followed by 6 ml of buffer C containing 100 mM α-methyl mannoside (warmed to 56°C). The material eluting with α-methyl mannoside was pooled, desalted, and treated with endo H for 18 hr to release the high-mannose-type oligosaccharides. The digest was passed over a column of Amberlite MB-3 (1 ml), concentrated, and chromatographed on Whatman no. 1 paper for 5 days. The paper was dried and cut into strips, and the ^{3}H radioactivity was determined.

Characterization of Glycopeptide-Bound [^{3}H]Mannose 6-Phosphate. The remaining 95% of the labeled glycopeptide material obtained from the Pronase digest was applied to Con-A Sepharose as described above, but all of the bound material was eluted directly with 100 mM α-methyl mannoside. The eluted material was pooled, desalted, treated with endo H for 18 hr, diluted to 2 ml with 2 mM Tris base, and applied to a column of QAE-Sephadex (3 × 0.5 cm) equilibrated in 2 mM Tris base. The column was washed with 6 ml of the same buffer and then eluted with 10 ml of 0.1 M HCl. Under these conditions, phosphorylated (and other negatively charged) oligosaccharides bound to the column and were eluted with the acid. The negatively charged oligosaccharides were hydrolyzed in 1 M HCl at 100°C for 4 hr. An internal standard of mannose 6-[^{32}P]phosphate was added to correct for losses during this and subsequent procedures.

The hydrolyzed material was dried under reduced pressure to remove the HCl, dissolved in 1 ml of water, and applied to a 1-ml column of Dowex AG-1-X8 (200–400 mesh; formate form). The column was washed successively with 3 ml of water, 6 ml of 0.1 M HCl, 6 ml of 0.2 M HCl, and 2 ml of 0.5 M HCl. The mannose 6-phosphate released from the oligosaccharides by hydrolysis was eluted in the 0.2 M HCl fraction. This fraction was dried under reduced pressure, spotted on Whatman no. 1 paper, and chromatographed for 12 hr. Strips (1 cm) were cut and the ^{3}H and ^{32}P radioactivities were determined. The mannose 6-phosphate peak was located, and the amounts of ^{3}H and ^{32}P in the peak tube were determined. The amount of [^{3}H]mannose 6-phosphate synthesized in high-mannose oligosaccharides during the pulse and chase periods was calculated and expressed as ppm of the total glycopeptide radioactivity.

Preparation of Fibroblast Homogenates. This was done exactly as described (19) except that the homogenization was carried out in 2 vol of buffer B.

Preparation of Fibroblast Membranes. Confluent cells were harvested and washed in 10 ml of buffer A. The pellet was resuspended in 2 vol of buffer A, sonicated (3 pulses, 15 sec each, Biosonik Mark IV microprobe at setting 50), and diluted to 10 ml with buffer A. After centrifugation at 165,000 × *g* for 30 min, the pellet was resuspended in buffer B (1.2×) with a Potter–Elvehjem homogenizer (Teflon pestle). Greater than 90% of the total GlcNAc*P*Tase activity in the sonicate was recovered in the membrane pellet.

Partial Purification of GlcNAc*P*Tase from Fibroblasts. All operations were carried out at 0–4°C. Fibroblasts were harvested and washed in 10 ml of buffer A. The pellet was resuspended in 2 vol of buffer A and sonicated as described above (except that each pulse was for 30 sec). The sonicate was centrifuged at 650 × *g* for 30 min, and the postnuclear supernatant was adjusted to final concentrations of 10 mg of protein per ml, 0.05% Triton X-100, and 400 mM NaCl, by using 10% Triton X-100, 4 M NaCl, and ice-cold distilled water. After vigorous vortexing (three times, 15 sec each), the suspension was centrifuged at 165,000 × *g* for 30 min. The resulting pellet was resuspended in buffer B (1.33×) (except for the final Triton X-100 concentration being 2.0%), in a volume that was equal to half that of the starting sonicate. After vigorous vortexing (three times, 15 sec each) the suspension was kept on ice for 12 hr and then centrifuged at 165,000 × *g* for 30 min. The resulting supernatant contained 60–75% of the GlcNAc*P*Tase activity present in the original sonicate and represented a 6- to 7-fold purification.

Assay of GlcNAc*P*Tase Activity. The transfer of GlcNAc 1-[^{32}P]phosphate from [β-^{32}P]UDP-GlcNAc to the 6-hydroxyl of mannose residues was studied in several different ways.

(*i*) Transfer to endogenous glycoprotein acceptors. This assay was performed with total fibroblast homogenates exactly as described (19). The specific activity of the UDP-GlcNAc was 800 cpm/pmol.

(*ii*) Transfer to exogenous α-methyl mannoside acceptor. This assay will be described in further detail elsewhere. The reaction conditions were identical to those used for the endogenous acceptor assay (15), except that 500 μg of fibroblast membrane or homogenate protein was used and the reaction mixture contained 100 mM α-methyl mannoside and 150 μM [β-^{32}P]UDP-GlcNAc (40–50 cpm/pmol) in a final volume of 50 μl. The reaction was quenched with 50 μl of 40 mM EDTA, the mixture was boiled for 5 min, diluted to 1 ml with 2 mM Tris base, and centrifuged for 5 min, and the supernatant was applied to a column of QAE-Sephadex (0.5 × 3 cm). The column was washed

with 4 ml of 2 mM Tris base, and the product (α-methyl mannoside 6-[^{32}P]*P*GlcNAc) was selectively eluted with 6 ml of 30 mM NaCl/2 mM Tris base and assayed for radioactivity. One unit of activity is defined as the transfer of 1 pmol of GlcNAc 1-*P* per hr. Serial studies during the early growth phase of the fibroblast cultures showed a 2-fold increase in activity from day 2 to day 7 after passage; thereafter, the activity remained relatively constant for 3 days before starting to decline. All assays were therefore performed at 7–10 days after passage.

(*iii*) Transfer to the mannose residues of high-mannose-type oligosaccharides. These assays were carried out as described above, with the endo H-released oligosaccharides from IgM in the place of α-methyl mannoside. Also, the reaction volume was decreased to 15 μl and the product ([^{32}P]*P*GlcNAc-Man$_{(5-8)}$-GlcNAc) was selectively eluted from the QAE-Sephadex column with 6 ml of 20 mM NaCl/2 mM Tris base. For these assays, 10 μl of the partially purified enzyme (see above) was used.

(*iv*) Transfer to added β-hexosaminidase A. Reaction mixtures (80 μl) contained buffer B, 50 mM GlcNAc, 15 μM [β-^{32}P]UDP-GlcNAc (1700 cpm/pmol), 5 mM ATP, 12 μg of human placental β-hexosaminidase A, and 50–150 μg of the partially purified enzyme protein. The GlcNAc was added to inhibit destruction of the UDP-GlcNAc. After incubation for 1 hr at 37°C, the proteins were precipitated by addition of 9 vol of ice-cold acetone. The pellet was washed once in 90% acetone, dissolved in 1% 2-mercaptoethanol/5% NaDodSO$_4$, and subjected to NaDodSO$_4$/polyacrylamide gel electrophoresis as described above. The gels were stained, fixed, dried, and then studied by autoradiography in order to detect transfer of ^{32}P to the oligosaccharides of specific proteins. Under these conditions, radioactive bands corresponding to β-hexosaminidase A subunits could be visualized in addition to several other bands representing endogenous glycoprotein acceptors.

RESULTS

Distinction Between I-Cell Disease and Pseudo Hurler Polydystrophy. Membranes from normal, I-cell, and pseudo Hurler polydystrophy fibroblasts were assayed for GlcNAc-*P*Tase activity with α-methyl mannoside as acceptor. The results are shown in Fig. 1, along with the results obtained with the previously reported endogenous acceptor assay (19). It is evident that, with the improved ability to detect low levels of enzyme activity, I-cell disease patients could now be distinguished from the pseudo Hurler patients (<0.4–2.0 units/mg vs. 2.9–39.4 units/mg, respectively). This finding may explain the difference in clinical severity between the two syndromes (27).

Identification of a Variant Form of Pseudo Hurler Polydystrophy. In contrast to the results obtained with all the other cases of pseudo Hurler polydystrophy, two siblings (GM 3391 and GM 3392) showed the unexpected combination of normal activity with the exogenous α-methyl mannoside assay and markedly decreased activity with the endogenous acceptor assay (Fig. 1).

Although these fibroblasts had the physical appearance and the low intracellular lysosomal enzyme levels characteristic of pseudo Hurler polydystrophy, we wished to confirm that they were indeed deficient in the phosphorylation of lysosomal enzymes in the intact cell. To do this, we measured the total gly-

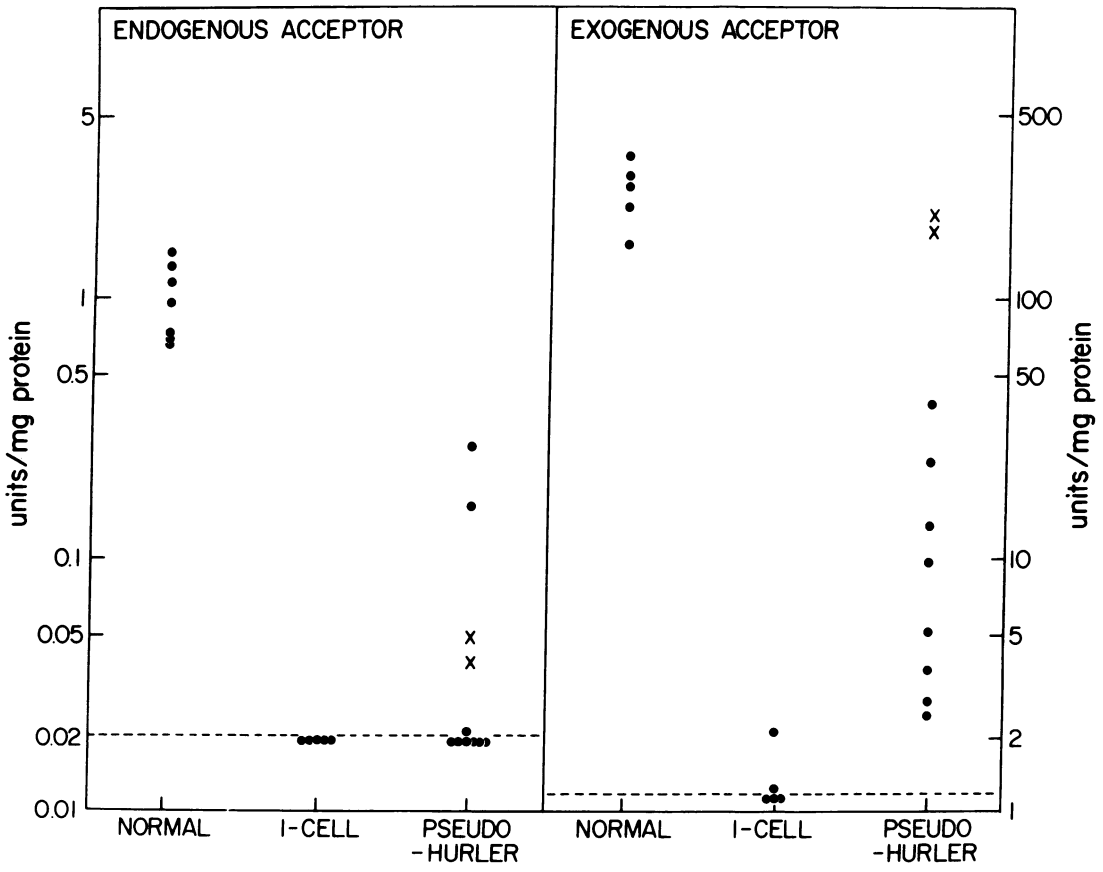

FIG. 1. GlcNAc*P*Tase activity in human fibroblasts assayed with either endogenous glycoprotein acceptors (*Left;* ref. 19) or exogenous α-methyl mannoside acceptor (*Right;* this work). Each point represents the mean of two to four determinations. The individual patients studied (●) were (from top to bottom, *Right*): pseudo Hurler, GM 113, GM 1494, GM 2559, GM 2558, GM 2065, GM 3685, GM 2425, and GM 1759; I-cell, GM 2933, GM 2273, GM 2013, GM 3066, and T.M. x, Values obtained with GM 3391 and GM 3392; broken lines, limits of detection of each assay.

coprotein [^{3}H]mannose 6-phosphate (in high-mannose-type oligosaccharides) synthesized by these cells during a short pulse with [^{3}H]mannose. This value was markedly decreased (620 ppm in GM 3391, and 630 ppm in GM 3392) compared to the levels in normal fibroblasts (2482, 4374, 4492, and 4480 ppm in four separate normal cell lines studied under identical conditions). This finding demonstrated that the GlcNAc*P*Tase was functionally deficient in the intact cell, in spite of the normal values obtained with the homogenate in the α-methyl mannoside assay.

We considered the possibility that the reason for the deficient phosphorylation of lysosomal enzymes in these cells might be an abnormality in the oligosaccharide units of these enzymes. We therefore studied the structure of the high-mannose-type oligosaccharides synthesized by these cells during a short pulse with [^{3}H]mannose. Total cellular glycopeptides were prepared, and the high-mannose-type oligosaccharides were isolated and analyzed. The endo H-released oligosaccharides displayed a profile of $Man_9GlcNAc_1$, $Man_8GlcNAc_1$, and smaller species similar to that seen in normal cells. Furthermore, all of these oligosaccharides could be degraded to Manβ-GlcNAc and free mannose by jack bean α-mannosidase. These findings indicate that most of the high-mannose oligosaccharides synthesized by these cells are normal. However, these data do not exclude a defect that affects lysosomal enzyme oligosaccharides selectively.

The kinetic properties of the GlcNAc*P*Tase obtained from the mutant fibroblasts were also compared to those of the normal fibroblast enzyme. The apparent K_ms for α-methyl mannoside, UDP-GlcNAc, and high-mannose-type oligosaccharides were similar for both enzymes (Table 1). Moreover, the K_m for the oligosaccharide was similar to that for the artificial acceptor, α-methyl mannoside.

Demonstration of the Defect in GM 3391 and GM 3392. The above data are most consistent with the abnormality in the mutant fibroblasts being an inability of the GlcNAc*P*Tase to recognize lysosomal enzymes as specific substrates for phosphorylation. We therefore tested the ability of the enzyme to transfer GlcNAc 1-*P* to the oligosaccharide units of purified placental β-hexosaminidase A.

Partially purified GlcNAc*P*Tase was incubated with [β-^{32}P]UDP-GlcNAc in the presence and absence of added β-hexosaminidase A, and the transfer of GlcNAc 1-[^{32}P]*P* to both endogenous glycoprotein acceptors and to exogenous β-hexosaminidase A was determined by subjecting the reaction products to polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$ (Fig. 2). Lane 1 demonstrates the phosphorylation of endogenous acceptor glycoproteins by the normal enzyme; lane 2 shows, in addition, the phosphorylation of the subunits of added β-hexosaminidase A. Lanes 3 and 4 show the results of similar incubations carried out with enzyme from GM 3392 fibroblasts. There was no significant phosphorylation of either the endogenous acceptors or the added β-hexosaminidase A in spite of the addition of an equal amount of catalytic activity. When the two extracts were mixed, the activity expected of the normal enzyme was seen (lane 5), indicating that the extract from GM 3392 did not contain an inhibitor. The large band of radioactivity that migrated close to the dye front disappeared completely in the presence of tunicamycin, indicating that it represents an independent reaction that is due to dolichol-*P*[^{32}P]*P*-GlcNAc synthesis (data not shown). Tunicamycin has no effect on the activity of GlcNAc*P*Tase (15, 20).

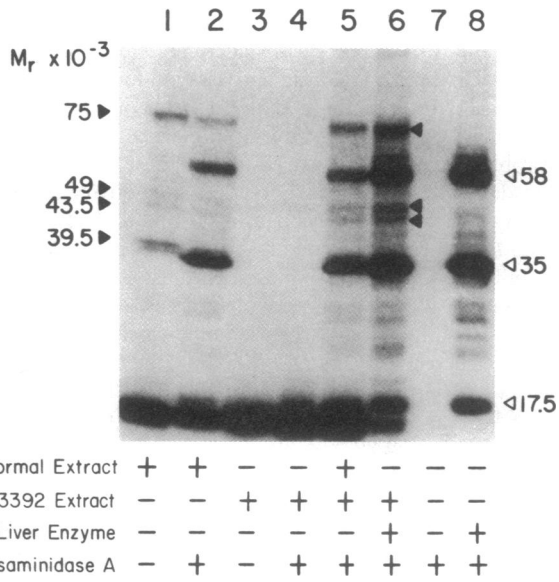

FIG. 2. Autoradiogram of $NaDodSO_4$/polyacrylamide gel electrophoresis. The reaction mixtures contained the various components as follows: normal fibroblast extract, 84 μg of protein, 100 units of activity; GM 3392 fibroblast extract, 140 μg of protein, 100 units of activity (except in lane 5, 42 μg of protein and 30 units of activity and in lane 6, 70 μg of protein and 50 units of activity); rat liver enzyme 20.8 μg of protein, 110 units of activity; and human placental β-hexosaminidase A, 12 μg of protein. The units of catalytic activity were determined with the α-methyl mannoside acceptor assay. After incubation for 1 hr at 37°C, the proteins were precipitated, washed, solubilized, and subjected to electrophoresis. Open arrows, three major bands resulting from phosphorylation of added β-hexosaminidase A; closed arrows, major endogenous acceptors in the fibroblast extracts.

Table 1. Apparent affinity constants of GlcNAc*P*Tase for various substrates

Substrate	Apparent K_m, mM: Normal fibroblasts	Apparent K_m, mM: GM 3392 fibroblasts
α-Methyl mannoside	78	109
UDP-GlcNAc	0.03	0.035
High-mannose oligosaccharide	53	54

N-Acetylglucosaminylphosphotransferase was partially purified from normal and GM 3392 fibroblasts. The incubation mixtures contained 10–20 μl of enzyme (30–60 μg of protein). The transfer of GlcNAc 1-[^{32}P]*P* to the different acceptors was assayed with varying concentrations of α-methyl mannoside or IgM oligosaccharide (UDP-GlcNAc at 150 μM) or with varying concentrations of UDP-GlcNAc (α-methyl mannoside at 100 mM). Double-reciprocal plots ([1/*V*] versus [1/*S*] of multiple data points obtained in duplicate showed a straight-line relationship in each case. The Michaelis–Menten constant for each substrate was derived from these plots.

When rat liver enzyme was incubated with β-hexosaminidase A, bands due to phosphorylation of the lysosomal enzyme subunits were seen (lane 8). In addition, there were some fainter bands from phosphorylation of endogenous acceptors. Addition of the rat liver enzyme to extracts of GM 3392 resulted in phosphorylation of the endogenous acceptors of the fibroblast extract (compare lane 6 to lane 8). This finding demonstrates that the GM 3392 fibroblasts have the same (or similar) endogenous acceptor proteins as are found in normal fibroblasts. In other experiments we were unable to demonstrate complementation when extracts from GM 113 fibroblasts (typical pseudo Hurler, with low catalytic activity) were mixed with extracts from GM 3391 (data not shown).

DISCUSSION

I-cell disease (mucolipidosis II) and pseudo Hurler polydystrophy (mucolipidosis III) are rare autosomal recessive lysosomal storage diseases. Both disorders are characterized by decreased

intracellular levels of many lysosomal enzymes, which results from failure to target these enzymes to their normal destination in the lysosomes (27). We (19) and others (20) have previously shown that these abnormalities are caused by a deficiency of GlcNAc*P*Tase, the first enzyme in the sequence that generates the common phosphomannosyl recognition marker of lysosomal enzymes. I-cell disease is the more severe of the two clinically, being recognized at birth and generally fatal in early childhood; pseudo Hurler polydystrophy has a later onset and a milder clinical course. The present data suggest that this clinical difference may be explained on the basis of the relative degree of deficiency of the GlcNAc*P*Tase. In all cases of I-cell disease, enzyme activity is extremely low or undetectable whereas in pseudo Hurler disease the activities are 2–20% of normal. The wide range of values seen in the pseudo Hurler patients may explain some of the variability in the clinical severity of this disease (28).

However, fibroblasts from two siblings (GM 3391 and GM 3392) with pseudo Hurler polydystrophy were found to have a variant form of GlcNAc*P*Tase deficiency. Although the catalytic activity of the enzyme appeared to be normal when assayed with an exogenous monosaccharide acceptor, there was deficient phosphorylation of endogenous proteins, both *in vitro* and in the intact cell. We were able to demonstrate that this is not due to an abnormality of the endogenous acceptors but rather results from failure of the transferase to recognize lysosomal enzymes as specific substrates for phosphorylation.

Because lysosomal enzymes are the only glycoproteins known to contain mannose 6-phosphate, there must be a mechanism by which these enzymes are selectively phosphorylated and thus marked for transport to the lysosomes. This could be accomplished either by selective compartmentalization of this group of proteins to a specialized subcellular region where the GlcNAc*P*Tase resides or by a recognition mechanism that makes them superior substrates for the transferase. The data obtained with the GM 3391 and 3392 fibroblasts strongly support the latter possibility. Our recent studies (29) with partially purified GlcNAc*P*Tase from rat liver also indicate that the latter possibility is the correct one. The catalytic efficiency (V_{max}/K_m) of the GlcNAc*P*Tase for the phosphorylation of several lysosomal enzymes was found to be greater than 100-fold higher than for nonlysosomal glycoproteins bearing high mannose-type oligosaccharides. The GlcNAc*P*Tase thus appears to have a recognition mechanism for selective phosphorylation of lysosomal enzymes.

The identification of this variant form of pseudo Hurler polydystrophy demonstrates the biological importance of this recognition mechanism in the generation of the common phosphomannosyl marker of lysosomal enzymes and provides a model for its further study.

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