Lysosomal Enzyme Phosphorylation in Human Fibroblasts

Kinetic Parameters Offer a Biochemical Rationale for Two Distinct Defects in the Uridine Diphospho-*N*-acetylglucosamine:Lysosomal Enzyme Precursor *N*-Acetylglucosamine-1-phosphotransferase

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Abstract

The primary genetic defect in the lysosomal storage disease mucolipidosis III (ML III) is in the enzyme uridine diphospho-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase. This enzyme has two well-defined functions: specific recognition of lysosomal enzymes (recognition function) and phosphorylation of their oligosaccharides (catalytic function). Using fibroblasts from patients with ML III as the source of enzyme, and α-methylmannoside and two lysosomal enzymes as the substrates, we have identified defects in both of these functions. In one group of fibroblasts, the catalytic activity of the *N*-acetylglucosaminylphosphotransferase is decreased while the ability to recognize lysosomal enzymes as specific substrates remains intact. In the second group of fibroblasts, the ability to recognize lysosomal enzymes is impaired while the catalytic activity of the enzyme is normal. These data provide a biochemical rationale for the previously described genetic heterogeneity among patients with ML III (Honey, N. K., O. T. Mueller, L. E. Little, A. L. Miller, and T. B. Shows, 1982, *Proc. Natl. Acad. Sci. USA.*, 79:7420–7424).

Introduction

Newly synthesized lysosomal enzymes acquire phosphomannosyl residues that allow specific binding to a receptor and selective targeting of these glycoproteins to lysosomes (reviewed in reference 1). Two enzymes are responsible for generating this marker: uridine diphospho-*N*-acetylglucosamine (UDP-GlcNAc):lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase (abbreviated *N*-acetylglucosaminylphosphotransferase)[1] and α-*N*-acetylglucosamine-1-phosphodiester *N*-acetylglucosaminidase. The first enzyme has been shown to recognize lysosomal enzymes as specific substrates in preference to nonlysosomal glycoproteins (2–4). This recognition is mediated by a protein determinant that is shared by many lysosomal enzymes (4). Based on these data it has been postulated that *N*-acetylglucosaminylphosphotransferase contains a catalytic site for mannose phosphorylation as well as a second site that mediates the specific recognition of the common protein determinant of lysosomal enzymes (4).

Mucolipidosis III (ML III) is characterized by decreased activities of many lysosomal enzymes in connective tissue cells, and by elevated levels of the same enzymes in body fluids (5). Fibroblasts from patients with ML III have defects in *N*-acetylglucosaminylphosphotransferase (6–8). The most common defect is a decrease in the total catalytic activity of the enzyme. However, a variant (GM3391) has been described that has normal levels of catalytic activity toward the substrate α-methylmannoside, but an inability to phosphorylate lysosomal enzymes under the conditions tested (7). It was concluded that the defect in this variant enzyme was in its ability to recognize lysosomal enzymes as specific substrates, rather than in its catalytic function. However, no kinetic studies were performed to substantiate this claim. Subsequently, Honey et al. (9) performed cell fusion experiments among different ML III fibroblast lines and found that these lines could be placed into two distinct complementation groups (with the possible existence of a third group) based on the partial restoration of lysosomal enzyme activities in the heterokaryons. GM3391 was assigned, along with four other ML III fibroblast lines, to one of these complementation groups (designated IIIC), and seven ML III fibroblast lines were assigned to the two other complementation groups (IIIA and IIIB).

The goal of the present study was to determine the kinetic parameters of *N*-acetylglucosaminylphosphotransferase from fibroblasts of ML III patients. In particular, we investigated whether the enzyme of complementation group IIIC patients retained any ability to recognize lysosomal enzymes in preference to nonlysosomal glycoproteins and, if so, whether the phosphorylation of all lysosomal enzymes was equally affected. The latter point is significant in terms of trying to understand the structural basis for the common protein determinant of lysosomal enzymes. The second question was whether the mutations in the other patients (presumed, but not proven, to represent complementation groups IIIA and IIIB) solely affected the catalytic activity of the *N*-acetylglucosaminylphosphotransferase, or if it also affected the ability of this enzyme to recognize lysosomal enzymes. It was felt that these data might provide a biochemical basis for the genetic heterogeneity that has been observed among patients with ML III.

Methods

Fibroblasts. Cultured human fibroblasts were supplied by the following individuals: L.A. (Dr. Larry Shapiro, University of California at Los Angeles Medical Center), E.V. (Dr. William Nyhan, University of California at San Diego), J.J. and M.R. (Dr. George Thomas, John F. Kennedy Institute), S.Z. and K.Z. (Dr. Mary Ella Pierpont and Dr. Chester

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Received for publication 24 May 1985.

1. *Abbreviations used in this paper:* ML III, mucolipidosis III; *N*-acetylglucosaminylphosphotransferase, lysosomal enzyme precursor *N*-acetylglucosamine-1-phosphotransferase; UDP-GlcNAc, uridine diphospho-*N*-acetylglucosamine.

J. Clin. Invest.

0021-9738/85/12/2191/05 $1.00
Volume 76, December 1985, 2191–2195

Whitley, University of Minnesota, Minneapolis, MN), T.A. and T.R. (Dr. Arnold Miller, University of California at San Diego), FB_2 and LF_{10} (Dr. Nancy Baenziger, Washington University Medical Center, St. Louis, MO), CRL-1224 and 82783 (Dr. Eugene Bauer, Washington University Medical Center), GM2987, GM3391, and AG1518 (Human Genetic Mutant Cell Repository, Camden, NJ). Cultures were maintained and harvested as previously described (6). Cell lysates from 2 to 4 P150 plates were prepared by sonication with a Biosonik IV microprobe (Bronwill Scientific, Rochester, NY) in 2 vol of 0.75% Lubrol, 50 mM Tris-HCl, pH 7.4, 1 mM $CaCl_2$, 1 mM $MgCl_2$. Additionally, lysates contained a 1:100 dilution of an antiprotease cocktail which consisted of aprotinin (11.5 TIU/ml) and chymostatin, pepstatin, leupeptin, and antipain (each at 2 mg/ml) in 50% dimethyl sulfoxide. Protein determination was by the method of Lowry et al. (10).

[β-^{32}P]UDP-GlcNAc. Synthesis was carried out exactly as described (11). When lysosomal enzymes were used as acceptors, the nucleotide sugar that had been eluted from the silica thin-layer chromatography plate was used directly. When α-methylmannoside was the acceptor, the nucleotide sugar was carried through an additional chromatographic step on Whatman paper (Whatman Chemical Separation, Inc., Clifton, NJ) in 1 M ammonium acetate (pH 3.8)/ethanol (7:3). This was done to decrease the background radioactivity in the assay.

Assays. Two assays for *N*-acetylglucosaminylphosphotransferase were used, as described in detail in reference 12. Briefly, these assays measure transfer of GlcNAc-[^{32}P] from [β-^{32}P]UDP-GlcNAc to mannose residues on oligosaccharide units of lysosomal enzymes or to the methylglycoside, α-methyl-D-mannoside. Reactions were carried out in a final volume of 50 μl. When the acceptor was α-methylmannoside, the [β-^{32}P]UDP-GlcNAc was present at 1×10^6 cpm in a final concentration of 150 μM. The phosphorylated methylglycoside was isolated by adsorption to and selective elution from QAE-Sephadex. Controls containing α-methylgalactoside (a nonacceptor) yielded a background of ~50 cpm. When lysosomal enzymes were used as the acceptors, the [β-^{32}P]UDP-GlcNAc was present at 3×10^6 cpm in a final concentration of 100 μM. The phosphorylated oligosaccharides were isolated on Con A-Sepharose after Pronase digestion, and the Con A–bound radioactivity was counted directly. Control reaction mixtures containing no exogenous acceptor yielded a background of ~100 cpm. Both assays typically resulted in the incorporation of several hundred to several thousand cpm of [^{32}P] into the final product.

Acceptors. Porcine spleen cathepsin D was prepared as previously described (13). Porcine uteroferrin (acid phosphatase) was generously supplied by Dr. R. Michael Roberts, University of Florida, Gainesville, FL. α-Methylmannoside was purchased from Sigma Chemical Co., St. Louis, MO, and α-methylgalactoside was purchased from Pfanstiehl Laboratories, Inc., Waukegan, IL.

Results

N-Acetylglucosaminylphosphotransferase assays. The *N*-acetylglucosaminylphosphotransferase assays were carried out under similar conditions regardless of whether α-methylmannoside or lysosomal enzymes were used as acceptors. The different concentrations of the nucleotide sugar (150 μM for α-methylmannoside and 100 μM for lysosomal enzymes) were well above the previously determined apparent Michaelis constant (K_m) of 30 μM (7). These conditions resulted in product formation that increased linearly with incubation time and was proportional to the amount of cell lysate added (data not shown). Fig. 1 shows representative double reciprocal plots for the three different substrates. Data such as these were used to calculate the apparent K_m and maximum velocity (V_{max}) values which are summarized in Table I.

Kinetics of N-acetylglucosamine-1-phosphate transfer by normal fibroblasts. Six fibroblast lines from unaffected individuals were analyzed to establish kinetic parameters for normal

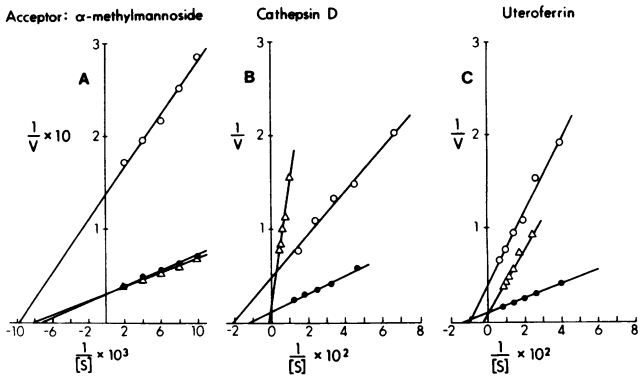

Figure 1. Kinetics of phosphorylation as a function of acceptor concentration. Representative data from assays using normal and ML III fibroblasts. Reactions contained between 119 and 387 μg of cell lysate protein. However, values used for this figure have been normalized to 240 μg (*A* and *B*) and 167 μg (*C*) to facilitate the comparison of data among the fibroblasts from groups A, B, and C of Table I. [S] = millimolars for α-methylmannoside and micromolars for the two lysosomal enzymes. V = picomoles of GlcNAC-P transferred/h. ●, AG1518 (normal from group A, Table I); ○, J. J. (ML III from group B, Table I); △, GM3391 (ML III from group C, Table I).

N-acetylglucosaminylphosphotransferase. As shown in Table I, the apparent K_m and V_{max} values obtained for the three acceptor substrates were consistent among the lines. These values were also similar to those obtained with a partially purified preparation of *N*-acetylglucosaminylphosphotransferase from rat liver, e.g., an apparent K_m for lysosomal enzymes in the micromolar range, and an apparent K_m for α-methylmannoside in the 100-mM range (4).

Kinetics of N-acetylglucosamine-1-phosphate transfer by ML III fibroblasts. A total of 18 ML III fibroblast lines were screened to identify 8 lines with sufficient *N*-acetylglucosaminylphosphotransferase activity to allow detailed kinetic studies. The results obtained with these eight fibroblast lines are summarized in Table I. These data fall into two distinct groups. Five fibroblast lines exhibited decreased enzyme activity toward all three substrates, as revealed by the low V_{max} values (Table I and ML III group, untested). With each of these lines, the affinity of the residual *N*-acetylglucosaminylphosphotransferase for α-methylmannoside and the two lysosomal enzymes was within the normal range. These data indicate that the residual *N*-acetylglucosaminylphosphotransferase in these fibroblasts is able to recognize lysosomal enzymes as specific substrates with an apparently normal affinity.

In contrast, fibroblast extracts from three of the patients displayed apparently normal kinetic parameters only when measured with α-methylmannoside (Table I, ML III group C). Though patient T.R. had a lower level of activity, the apparent V_{max} was actually 80% of the lowest observed normal value, placing it closer to the normal values than to those for the ML III patients described in Table I (ML III group, untested). These three patients have previously been assigned to complementation group IIIC of ML III patients (9). Each of these fibroblast extracts could phosphorylate both of the lysosomal enzymes, but the affinity for these acceptors was greatly decreased. In two instances it was possible to test concentrations of lysosomal enzymes near the K_m, allowing the determination of an apparent K_m value of 500 μM for cathepsin D (T.R.) and a value of 244 μM for uteroferrin (T.A.). In both of these cases, the apparent V_{max} values

Table I. Kinetics of GlcNAc-Phosphate Transfer by N-acetylglucosaminylphosphotransferase from Normal and ML III Fibroblasts

	α-Methylmannoside		Cathepsin D		Uteroferrin	
Cell line	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
Normal (group A)						
82783	89,000	355	72	120	115	161
AG1518	136,000	273	82	36	73	59
CRL-1224	73,000	495	52	86	112	154
GM-2987	116,000	408	68	51	51	75
L.F.$_{10}$	86,000	446	56	118	88	147
L.A.	117,000	397	ND	ND	ND	ND
F.B.$_2$	60,000	285	59	108	91	127
Average	97,000	380	65	87	88	121
ML III group untested (group B)						
E.V.	159,000	40 (11)	56	3 (3)	47	9 (7)
J.J.	103,000	60 (16)	47	9 (10)	102	16 (13)
M.R.	73,000	59 (16)	95	11 (13)	106	19 (16)
S.Z.	180,000	53 (14)	76	10 (11)	98	19 (16)
K.Z.	75,000	69 (18)	58	16 (18)	72	23 (19)
ML III (group C)						
GM3391	127,000	274 (72)	1652	*	417	*
T.A.	63,000	401 (106)	9396	*	244	137 (113)
T.R.	109,000	218 (57)	500	82 (94)	1283	*

All values were generated from double reciprocal plots as shown in Fig. 1. The apparent K_m values are given as micromolar concentrations, while the V_{max} values are given as picomoles GlcNAc-P transferred/h per mg of fibroblast protein. The numbers in brackets represent the percent of residual activity found in ML III fibroblasts; i.e., [V_{max} (ML III)/V_{max} (normal average)]. * In these cases the apparent K_m values were so much greater than the highest substrate concentration tested that reliable V_{max} values could not be calculated.

were in the normal range. In the other cases the double reciprocal plots passed too close to the origin to yield reliable kinetic parameters, indicating that even the highest substrate concentration tested was well below the true K_m value.[2] Regardless, it is clearly evident that in these instances the affinity of the *N*-acetylglucosaminylphosphotransferase for lysosomal enzymes is markedly abnormal.

Relative catalytic efficiencies (V_{max} divided by apparent K_m) are generally used as a measure of how well a particular substrate can be utilized by an enzyme (14). The relative catalytic efficiencies for the various acceptors are shown in Table II, with the values normalized to α-methylmannoside. These data reveal that the *N*-acetylglucosaminylphosphotransferase from normal fibroblast lines phosphorylate lysosomal enzymes ~320-fold more efficiently than α-methylmannoside. The *N*-acetylglucosaminylphosphotransferase from the five ML III fibroblast lines that exhibited decreased activity gave similar values, consistent with the residual enzyme in these cells being able to recognize lysosomal enzymes as well as the normal *N*-acetylglucosaminylphosphotransferase. The enzyme from the three ML III fibroblast lines that had normal activity only toward α-methylmannoside phosphorylated lysosomal enzymes much less efficiently compared with the other fibroblasts, but clearly acted upon lysosomal enzymes better than on α-methylmannoside. This indicates that these mutant enzymes retain some capacity to recognize lysosomal enzymes as specific substrates.

2. Due to both a limited supply of lysosomal enzyme substrate and solubility problems at high concentrations, it was not possible to use higher levels of substrate in these assays.

As an additional test, the relative catalytic efficiency for ribonuclease B, a nonlysosomal glycoprotein, was determined using GM3391 fibroblasts. A value of 1.9 was obtained, compared with 13 for cathepsin D and 79 for uteroferrin. This provides further evidence that the *N*-acetylglucosaminylphosphotransferase from complementation group IIIC cell lines has some ability to recognize lysosomal enzymes.

Discussion

The enzyme *N*-acetylglucosaminylphosphotransferase catalyzes the transfer of *N*-acetylglucosamine-1-phosphate from UDP-GlcNAc to α-linked mannose residues, including α-methylmannoside, high mannose oligosaccharides, and glycoproteins bearing high mannose oligosaccharides (2). The enzyme also functions to specifically recognize some protein determinant common to many lysosomal enzymes, thereby reducing the apparent K_m of these glycoproteins to the low micromolar range and allowing their selective phosphorylation (4). These two functions define two distinct sites (or subunits) of the enzyme: a catalytic one and a recognition one. These functionally distinct sites, however, need not be spatially separated.

A deficiency in *N*-acetylglucosaminylphosphotransferase activity is the primary defect in patients with the lysosomal storage diseases mucolipidosis II and mucolipidosis III (6, 15, 16). Fibroblasts from patients with ML II have low or undetectable enzyme levels, whereas fibroblasts from patients with ML III have residual phosphorylating activity (7, 8), consistent with their milder clinical course (17). Cell fusion experiments have defined complementation groups among various ML II and ML III fi-

Table II. Catalytic Efficiencies for Three Substrates Phosphorylated by N-acetylglucosaminylphosphotransferase from Normal and ML III Fibroblasts

Cell line	α-Methylmannoside	Cathepsin D	Uteroferrin
Normal			
82783	1	418	351
AG1518	1	218	402
CRL-1224	1	244	203
GM-2987	1	213	418
$L.F._{10}$	1	406	322
$F.B._{2}$	1	385	294
Average	1	314	332
ML III group untested			
E.V.	1	214	766
J.J.	1	330	270
M.R.	1	143	221
S.Z.	1	454	669
K.Z.	1	300	347
ML III group C			
GM3391	1	13*	79*
T.A.	1	21*	88
T.R.	1	82	78*

Catalytic efficiency is defined as V_{max} divided by apparent K_m. Data from Table I have been used to generate these values, which have been normalized to α-methylmannoside = 1.

* These values are equal to the inverse of the slopes of the respective double reciprocal plots (slope = K_m/V_{max}) and may be less reliable than the other values since substrate concentrations were below the apparent K_m.

broblast lines (9, 18). The ML III lines have been placed into two distinct complementation groups, with the possible existence of a third. The group IIIA (and IIIB) fibroblasts have decreased *N*-acetylglucosaminylphosphotransferase activity toward α-methylmannoside whereas the group IIIC fibroblasts contain enzyme with normal or nearly normal levels of activity toward that substrate. Complementation group IIIC includes the variant GM3391, which was previously concluded to have a defect in recognition of lysosomal enzymes (7). However, a kinetic analysis of lysosomal enzyme phosphorylation was not done in any of these studies.

Using two purified lysosomal enzymes, cathepsin D and uteroferrin, we have characterized the kinetics of phosphorylation by fibroblast extracts from eight ML III patients. Fibroblasts from five patients displayed decreased activity toward both lysosomal enzymes and α-methylmannoside, while the apparent K_m values for all three substrates were normal. Therefore, the residual *N*-acetylglucosaminylphosphotransferase must have an intact recognition function. The possible reasons for the low enzyme activity in these patients include decreased synthesis of the enzyme, instability of the enzyme, or synthesis of an enzyme with a defective catalytic function.

Fibroblast extracts of three patients (all from complementation group IIIC) displayed normal or nearly normal kinetic parameters when α-methylmannoside was the acceptor, suggesting the presence of a normal level of the enzyme. In cases where reliable double reciprocal plots could be generated, the level of enzyme activity was also shown to be normal using lysosomal enzymes as the acceptor. However, the affinity for the lysosomal enzymes was significantly decreased. Therefore, these patients appear to contain normal levels of *N*-acetylglucosaminylphosphotransferase which is defective in its recognition function.

These kinetic data establish two distinct types of biochemical defects in ML III patients, and provide a biochemical basis for the existence of two complementation groups (9). Since complementation in somatic cells occurs at the protein level, the prior demonstration of at least two complementation groups suggested that the *N*-acetylglucosaminylphosphotransferase is an oligomeric protein (9, 19). While it is not possible to distinguish between an oligomer of identical subunits or one with different, specialized subunits, these data suggest as a working model of the *N*-acetylglucosaminylphosphotransferase a protein that contains a recognition site (or subunit) and a catalytic site (or subunit), interacting to specifically recognize and phosphorylate lysosomal enzymes. Since complementation studies were not performed with the ML III fibroblasts that have decreased catalytic activity, it is possible that these patients do not fall into the complementation group(s) IIIA (IIIB) of Honey et al. (9). Nevertheless, it seems extremely likely that they are in a complementation group other than group IIIC.

While the *N*-acetylglucosaminylphosphotransferase from all three of the group IIIC fibroblast lines exhibited abnormally high K_m values for the two lysosomal enzymes, there were differences in the relative affinities for these two substrates. In patient T.A., the apparent K_m for uteroferrin was only modestly affected (2.8-fold increase) while the apparent K_m for cathepsin D was increased over 12-fold. By comparison, in patient T.R., the K_m for cathepsin D was less affected whereas the K_m for uteroferrin was more abnormal. This suggests that there may be some "wobble" in the protein recognition marker present on lysosomal enzymes, so that a defect in the recognition site of the *N*-acetylglucosaminylphosphotransferase could selectively affect recognition of some lysosomal enzymes compared with others. This difference in recognition of various lysosomal enzymes may account for the variable levels of residual lysosomal enzyme activities found in these fibroblasts (9). It could also contribute to the heterogeneity in the clinical phenotypes of ML III patients.

Of further note are the actual levels of residual activity in fibroblasts from some of the patients described in Table I (ML III group, untested). Obligate heterozygotes have intermediate levels of *N*-acetylglucosaminylphosphotransferase activity and are clinically unaffected, although they do exhibit increased serum β-hexosaminidase levels (16). Some of the ML III patients had residual activities as high as 18% of normal, and yet were clearly affected clinically, suggesting that the amount of *N*-acetylglucosaminylphosphotransferase is quite limiting, at least in some tissues.

Acknowledgments

The authors wish to thank Karen Fujimoto for superb technical assistance, and Laurie Bourisaw for assistance in preparation of this manuscript.

This work was supported by U. S. Public Health Service grants R01 CA 08759, 5T32GM 07067, and 5T32GM 07200.

References

1. Creek, K. E., and W. S. Sly. 1984. The role of the phosphomannosyl receptor in the transport of acid hydrolases to lysosomes. *In* Lysosomes

in Biology and Pathology. J. T. Dingle, R. T. Dean, and W. Sly, editors. Elsevier/North Holland, New York. 63–82.

2. Reitman, M. L., and S. Kornfeld. 1981. Lysosomal enzyme targeting: *N*-acetylglucosaminylphosphotransferase selectively phosphorylates native lysosomal enzymes. *J. Biol. Chem.* 256:11977–11980.

3. Waheed, A., A. Hasilik, and K. von Figura. 1982. UDP-*N*-acetylglucosamine:lysosomal enzyme precursor *N*-acetylglucosamine-1-phosphotransferase: partial purification and characterization of the rat liver Golgi enzyme. *J. Biol. Chem.* 257:12322–12331.

4. Lang, L., M. Reitman, J. Tang, R. M. Roberts, and S. Kornfeld. 1984. Lysosomal enzyme phosphorylation: recognition of a protein-dependent determinant allows specific phosphorylation of oligosaccharides present on lysosomal enzymes. *J. Biol. Chem.* 259:14663–14671.

5. McKusick, V. A., E. F. Neufeld, and T. E. Kelly. 1978. Two disorders of lysosomal enzyme localization: mucolipidosis II (ML II, I-cell disease, inclusion cell disease) and mucolipidosis III (ML III, pseudo-Hurler polydystrophy). *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill Book Co., New York. 1299–1302.

6. Reitman, M. L., A. Varki, and S. Kornfeld. 1981. Fibroblasts from patients with I-cell disease and pseudo-Hurler polydystrophy are deficient in uridine 5′-diphosphate-*N*-acetylglucosamine: glycoprotein *N*-acetylglucosaminylphosphotransferase activity. *J. Clin. Invest.* 67:1574–1579.

7. Varki, A. P., M. L. Reitman, and S. Kornfeld. 1981. Identification of a variant of mucolipidosis III (pseudo-Hurler polydystrophy): a catalytically active *N*-acetylglucosaminylphosphotransferase that fails to phosphorylate lysosomal enzymes. *Proc. Natl. Acad. Sci. USA.* 78:7773–7777.

8. Waheed, A., A. Hasilik, M. Cantz, and K. von Figura. 1982. Phosphorylation of lysosomal enzymes in fibroblasts: marked deficiency of *N*-acetylglucosamine-1-phosphotransferase in fibroblasts of patients with mucolipidosis III. *Hoppe-Seyler's Z. Physiol. Chem.* 363:169–178.

9. Honey, N. K., O. T. Mueller, L. E. Little, A. L. Miller, and T. B. Shows. 1982. Mucolipidosis III is genetically heterogeneous. *Proc. Natl. Acad. Sci. USA.* 79:7420–7424.

10. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

11. Lang, L., and S. Kornfeld. 1984. A simplified procedure for synthesizing large quantities of highly purified uridine [β-^{32}P]diphospho-*N*-acetylglucosamine. *Anal. Biochem.* 140:264–269.

12. Reitman, M. L., L. Lang, and S. Kornfeld. 1984. UDP-*N*-acetylglucosamine: lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase. *Methods Enzymol.* 107:163–172.

13. Takahashi, T., and J. Tang. 1981. Cathepsin D from porcine and bovine spleen. *Methods Enzymol.* 80:565–581.

14. Segal, I. H. 1976. Biochemical Calculations. John Wiley and Sons, New York. 208–219.

15. Hasilik, A., A. Waheed, and K. von Figura. Enzymatic phosphorylation of lysosomal enzymes in the presence of UDP-*N*-acetylglucosamine. Absence of the activity in I-cell fibroblasts. *Biochem. Biophys. Res. Commun.* 98:761–767.

16. Varki, A., M. L. Reitman, A. Vannier, S. Kornfeld, J. H. Grubb, and W. S. Sly. 1982. Demonstration of the heterozygous state for I-cell disease and pseudo-Hurler polydystrophy by assay of *N*-acetylglucosaminylphosphotransferase in white blood cells and fibroblasts. *Am. J. Hum. Genet.* 34:717–729.

17. Thomas, G. H., H. A. Taylor, L. W. Reynolds, and C. S. Miller. 1973. Mucolipidosis III (pseudo-Hurler polydystrophy): multiple lysosomal enzyme abnormalities in serum and cultured fibroblast cells. *Pediatr. Res.* 7:751–756.

18. Mueller, O. T., N. K. Honey, L. E. Little, A. L. Miller, and T. B. Shows. 1983. Mucolipidosis II and III. The genetic relationships between two disorders of lysosomal enzyme biosynthesis. *J. Clin. Invest.* 72:1016–1023.

19. Reusser, A. J. J. 1984. Genetic heterogeneity in lysosomal storage disorders studied by somatic cell hybridization. *In* Molecular Basis of Lysosomal Storage Disorders. J. A. Barranger and R. O. Brady, editors. Academic Press, London. 287–309.